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6844

Blood Sugar Studies Following Hypophysectomy and Experimental Lesions of Hypothalamus.*

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It is not clear whether the relation of hypophysis and hypothalamus to carbohydrate metabolism is due primarily to one or the other, or to an interaction between both. We wish to record the following progress report pertaining to the subject.

All operations were made under nembutal† by the subtemporal approach with excellent visibility and little or no hemorrhage.

Fasting Blood Sugars. In 45 normal dogs, the fasting blood sugars determined by a modified Folin-Wu method ranged from 75 to 139 mg. %, with only 2 cases below 80. We consider values of 70 and below abnormal. After complete hypophysectomy, abnormally low blood sugars were observed in 7 out of 11 cases, values of 34, 43, 59, 63, 69, and 70 being recorded. Removal of the anterior lobe alone in one specimen resulted in hypoglycemia (40). In each case, the low blood sugar was accompanied by symptoms typical of cachexia hypophysoprivea. These crises were spontaneous and varied in their onset from 1 day to 3 months postoperative. In 4 cases in which glucose was given, immediate recovery was effected; one animal still survives. In 2, symptoms were reprecipitated by a short period of starvation. These results are in exact agreement with those reported by Houssay and Biasotti.[1] Two of the remaining dogs died the first day postoperative without low blood sugars, one, after several weeks and one is still living after 3 months.

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* This investigation is being aided by a grant from the Committee on Scientific Research of the American Medical Association.

† Courtesy Abbott Laboratories, Chicago.

Hypoglycemic crises similar to those following hypophysectomy were observed in 5 out of 13 dogs with bilateral transverse lesions at the chiasmal level of the hypothalamus (68, 68, 50, 65, 68). These occurred on the first and second days and were followed by death in 4 cases; the fifth recovered on administration of glucose and maintained a normal blood sugar until death on the fifth day. Four of the remaining 8 retained normal blood sugars until terminated 1 to 2 months after operation. The other 4 died within the first 4 days without showing low blood sugars. It is possible that a hyperglycemia due to the excitement which is a characteristic result of these lesions overshadowed the hypoglycemic effect. Values from 150 to 166 occurred during periods of excitement.

No abnormally low fasting blood sugars appeared in 6 animals with bilateral transverse lesions at the middle of the hypothalamus; 4 with bilateral transverse prechiasmal lesions; 4 with ventral third of hypothalamus removed; 2 with maceration of the whole hypothalamus.

Hyperglycemia (except during excitement) did not occur, even transiently, except with complete removal of the hypothalamus and after transection of the brain stem caudal to the hypothalamus.

Glucose Tolerance was determined by analyzing blood samples at the ½, 1 and 2 hour intervals following administration of 2 gm. per kilo of glucose by stomach tube. In 26 determinations on 23 unoperated dogs, the distribution was: 6 low, never over 150; 15 normal, under 200, ending below 150; 4 high, above 200, but below 150 at end; 1 high prolonged, over 200 and ending above 150. Standards were chosen arbitrarily.

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[1] Houssay, B. A., and Biasotti, A., *Endocrinol.*, 1931, **15**, 511.

Tests made after operation (1-14 days) on 6 dogs with chiasmal lesions, 1 with ventral third of hypothalamus removed, 1 with anterior lobe removed, 1 with posterior lobe removed, and 6 with complete hypophysectomy, as well as 2 with hemorrhage at the base of the brain, but no experimental lesion, all showed high or high prolonged curves. In those dogs which could be followed, including one or more of each group, the curves returned to normal within from 9 to 20 days.

Starvation on Tolerance Curve. One chiasmal, 1 posterior lobe, 2 hypophysectomized dogs, and 1 with stalk detached, in all of which the tolerance curve had returned to normal, were starved, tests run at 4, 8, and 14 days, and the results compared with 3 starving, unoperated dogs. All gave high or high prolonged curves, but with these striking differences: At the end of the 4 and 8 day periods, blood sugars in the control and posterior lobe dogs were normal or only slightly high at the end of the second hour, while the other operated dogs were highest at this time (above 200). After 14 days of starvation, blood sugars of the control and posterior lobe animals were normal or falling at the end of the fourth hour, while those of the other operated dogs were higher than at any time earlier in the test (255-357). Curves were thus higher and more prolonged in the 4 operated than in the control and posterior lobe dogs at the same period of starvation. (Excessively high blood sugars lasting for from 4 to 6 hours were observed when glucose was given to dogs with hypoglycemia.)

Several of the dogs, in which the crises occurred several days or more after operation, exhibited marked degeneration of the liver. In 3 instances—2 hypophysectomy, 1 chiasmal—large duodenal ulcers were present which simulate experimental ulcers produced by ligation of the bile ducts.

Since it is impossible to duplicate lesions exactly, histological studies, now in progress, may modify groupings and explain discrepancies.

6845

Concerning the Mechanism of Contraction of the Gall-bladder in the Guinea Pig.

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After studying the evacuation of bile in the opossum and the cat by cholecystographic methods,[1] and having found that in the opossum contractions of the gall-bladder sufficiently great to be recorded by moving pictures may be induced by faradic stimulation of its wall,[2] it has seemed desirable to investigate anew the extent to which the biliary vesicle is under control of the nervous system. Bearing in mind the emphasis recently placed by Boyden[3] upon species differences in the reactions of mammalian gall-bladders, we have chosen to begin with the guinea pig—that species in which the gall-bladder appears to be more sensitive to a variety of stimuli than in any other vertebrate yet described.[4]

All experiments were conducted with the animal under nembutal anesthesia and with the liver gently everted over the costal margin. This routine exposure gave access to the gall-bladder, to the cystic and hepatic ducts and to the structures in the lesser omentum. A heart lever attached to the fundus of the gall-bladder recorded contractions of the vesicle on a smoked drum. To evaluate changes due to respiration and other extraneous movements, a second lever was attached to a bile duct near the hilus of the liver. The possibility of the spreading of the current to the wall of the gall-bladder was ruled out by the fact that faradic stimulation of the stomach, bowel, hepatic ducts and adjacent portions of the liver failed to cause contraction of the biliary vesicle.

With the apparatus thus arranged, marked contractions of the gall-bladder were recorded when the following were stimulated with a weak faradic current: (1) the viscus itself, (2) the duodenal portion (ampulla) of the common bile duct, (3) either vagus nerve in its cervical portion and (4) certain poorly defined cords of the lesser omentum posterior to the common bile duct. Following contraction induced by direct stimulation of its wall the gall-bladder usually relaxed to its previous tone level. After the contraction

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[1] DuBois, F. S., and Hunt, E. A., *Anat. Rec.*, 1932, **54**, 289.

[2] DuBois, F. S., and Kistler, G. H., *Anat. Rec.*, 1933, **55** (Suppl.), 14.

[3] Boyden, E. A., *Proc. Am. Physiol. Soc.*, 1933, **10**.

[4] Mann, F. C., and Higgins, G. M., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 931.

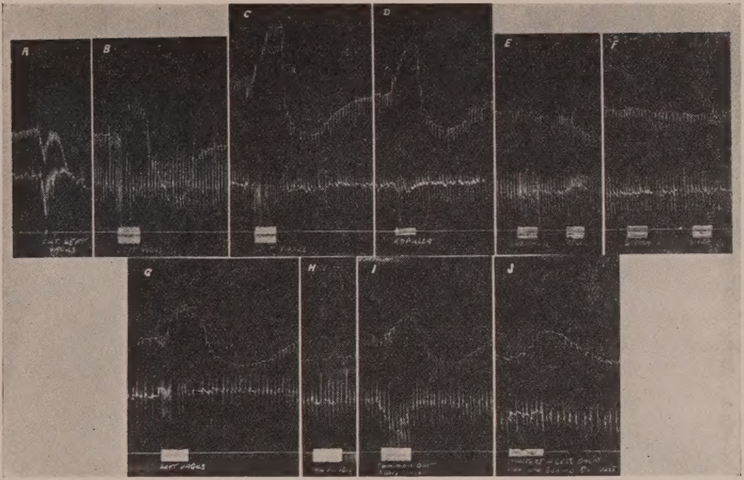

FIG. 1.

Contraction of the gall bladder. Upper tracing indicates tone of gall bladder. Lower tracing indicates movements extraneous to gall bladder (see text).

Left vagus nerve severed (A). Common bile duct intact; weak faradic current applied to: left vagus nerve (B and C), ampulla (D), duodenum and jejunum (E), and stomach and liver (F). Common bile duct severed; stimulation of: left vagus (G), ampulla (H), hepatic end of severed duct (I), and cords of lesser omentum (J).

which resulted from stimulation of the vagus, and to a less extent after that which resulted from stimulation of the ampulla, the tone of the gall-bladder dropped far below that which preceded contraction. A similar rapid diminution of tone was observed following division of one vagus nerve in the neck. When the common bile duct was severed, stimulation of the ampulla no longer caused contraction of the gall-bladder, but the latter responded in the usual way to stimulation of its wall, to excitation of either vagus nerve, and to stimulation of the hepatic end of the severed bile duct. In the absence of histologic examination this finding seems to point to the existence of a direct nervous pathway from ampulla to gall-bladder along the wall of the common duct—in addition to the usual plexus which accompanies the vessels of the duodeno-hepatic ligaments. Furthermore, since the severing of the bile duct did not eliminate contraction of the vesicle, it would appear that peristalsis of the ampulla is not so important a factor in the evacuation of the gall-bladder of the guinea pig as has been postulated.[5]

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[5] Burget, G. E., and Brocklehurst, R. J., *Am. J. Physiol.*, 1928, **83**, 578.

Lastly, it was observed that strong contractions of the gall-bladder could be induced by the direct application of pilocarpine to its wall. Atropine in small amounts, similarly applied, decreased the tone of the gall-bladder and rendered it refractory to pilocarpine, to excitation of the vagus nerves and to stimulation of the ampulla, but not to faradic stimulation of its wall. Adrenalin produced immediate and rather prolonged inhibition of the gall-bladder. This was present also after atropine.

In conclusion, the response of the gall-bladder to these autonomic drugs and to vagus stimulation suggests the existence in this organ of a thoracolumbar-parasympathetic nervous system antagonism—an hypothesis which is being subjected to further investigation.

6846

Studies on the Comparative Value of Intravenous Sclerosing Substances.

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Ochsner and Garside[1] reported the results of their studies on the effect of intravenous injection of 20 different sclerosing solutions. This paper is a report of the continuation of that work, comprising a similar study of 9 additional solutions.

Method. The methods used were the same as those previously employed. Eighteen dogs were used, and 4 injections were made at different levels in the veins of each leg, thus making 16 injections of a sclerosing substance in each dog. Only one drug was used for each leg, but different drugs were sometimes injected into each leg of the animal. Five-tenths cc. of each of the following solutions were injected into 16 veins:

	%		%
Searle's sodium morrhuate	—5	Sodium morrhuate (own)	—10, 5
Sodium gynocardate	—5, 3, 2	Sodium hydnocarpate	—5, 3, 2

The area of injection was marked and subsequently the veins were cautiously removed under local anesthesia to prevent the dislodgement of thrombi. The more distal segments of vein were removed first.

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[1] Ochsner, Alton, and Garside, Earl, *Ann. Surg.*, 1932, **96**, 691.

Sections were removed at the following intervals: ½, 1, 3, 6, 12, 24, and 48 hr., 3, 4, 6, 10, 14, 21 days, 4, 6, and 8 weeks. Thus, there were 16 sections studied for each solution.

After removal the veins were prepared for microscopic study by staining with hematoxylin and eosin and Mallory's connective tissue stains. Some sections were stained by Werhoff's elastic tissue method.

Thrombus Production. Searle's sodium morrhuate produced thrombosis in the greatest number of instances, 71.4% of the veins, sodium gynocardate (5%) in 50%, sodium gynocardate (3%) in 50%, sodium gynocardate (2%) in 44%, sodium hydnocarpate (5%) in 37.5%, sodium morrhuate (own, 5%) in 33%, sodium hydnocarpate (3%) in 33%, sodium morrhuate (10%) in 19%, and sodium hydnocarpate (2%) in 12.5%.

As the methods used were the same and as one* of us studied the sections in each series, our results may be compared with those of Ochsner and Garside. In comparing the thrombus producing effectiveness of the drugs in both series, we find that their most effective experimental thrombus producer was only fourth in the entire series. Their sodium salicylate, 40%, produced thrombosis in only 47% of the veins, whereas our 3 most effective thrombus producers showed 71.4%, 50%, and 50% thrombosis, respectively, *v.s.*

Endothelial Destruction. The grade and incidence of endothelial destruction closely parallels the incidence of thrombosis. We have performed other experiments *in vitro* with Searle's sodium morrhuate and quinine and urea hydrochloride to show that these 2 sclerosing solutions do not decrease the clotting time of blood. Thrombosis occurs after injection of sclerosing substances by destruction of endothelium and liberation of thrombokinase. Sodium gynocardate, 5%, showed some endothelial destruction in 75% of the sections, but complete destruction in only 25%; whereas Searle's sodium morrhuate produced complete destruction of the endothelium in 72% of the sections. The remainder of the 9 solutions used by us produced endothelial destruction in smaller percentages; the least effective, sodium hydnocarpate 2%, showed evidences of endothelial destruction in only 19% of the sections.

Other Changes. Various other changes were found in the vein wall: edema, leucocytic infiltration, destruction of the internal elastic lamina, coagulation necrosis, new vessels, muscle atrophy. These changes, inflammatory or reparative in nature, will be reported by us in detail elsewhere.

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* Alton Ochsner.

6847

Luteinization in the Immature Guinea Pig.*

ARTHUR G. KING. (Introduced by Harold Cummins.)

From the Department of Obstetrics, Tulane University Medical School.

In the investigation of the various sex hormones, the guinea pig has been neglected chiefly because it does not readily respond to manipulation. In the ovary of rats, mice, and rabbits, the effect of treatment with anterior pituitary-like substances is usually maturation of follicles, ovulation, and the formation of corpora lutea. No such changes have been reported for the guinea pig.

Jares[1] found that the ovaries of mature guinea pigs injected intravenously with pregnancy urine were entirely negative. Loeb and Kountz[2] reported that injection of ovarian extract produced in the immature ovary a slight acceleration of maturation. Aron[3] used anterior pituitary extracts and described a growth and vascularization of the theca interna and a degeneration of the granulosum cells leading to atresia. Almost the same picture was presented by Papanicolaou[4] using the urine of pregnant cows.

In the course of a study of the immature guinea pig the following significant observation was made; that luteinization can be produced in less than 48 hours by the injection of a suitable amount of anterior pituitary-like substance. The luteinization occurred in the ovaries of animals weighing from 205 to 265 gm. after injection of (a) pregnancy urine of high titre (3.0-4.0 cc.), (b) commercial anterior pituitary-like substances extracted from pregnancy urine (200 units), (c) sheep anterior pituitary gland emulsion (0.5-2.0 cc.) and (d) to a limited extent by pregnancy blood serum. True corpora lutea were not formed.

The steps in the process seem to be as follows: (1) a degeneration of the granulosum cells with an increased vascularity of the stroma and the theca interna; (2) an outpouring of a primitive type of cell resembling a fibroblast. These 2 steps were also produced by *theelin* alone, either by the direct action of *theelin* on the particularly sensitive ovarian cells or else by reflex stimulation of the pituitary gland of the animal. The third step is luteinization of the theca interna, and fourth, luteinization of undifferentiated cells throughout the whole ovary. (See figures.)

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* This work was aided by a grant from the David Trautman Schwartz Research Fund. Appreciation is expressed to Dr. Oliver Kamm of Parke, Davis and Co., for a supply of Antuitrin-S and Sheep Anterior Pituitary Gland Emulsion.

[1] Jares, J., *Anat. Rec.*, 1931, **49**, 185.

[2] Loeb, L., and Kountz, W. B., *Am. J. Phys.*, 1928, **84**, 283.

[3] Aron, M., *Compt. rend. Soc. Biol.*, 1931, **108**, 20.

[4] Papanicolaou, G., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 807.

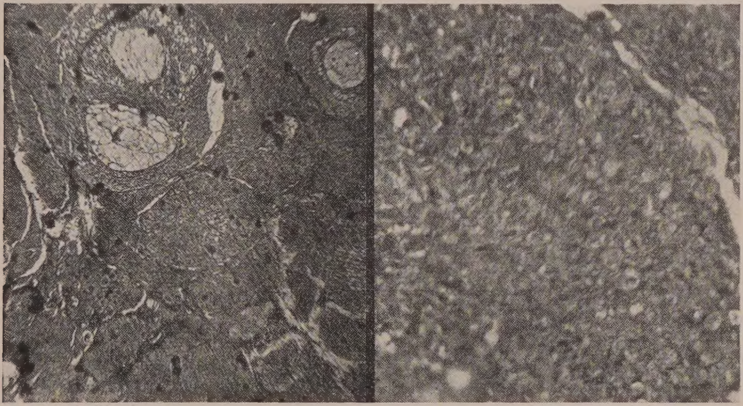

FIG. 1. FIG. 2.

Luteinization in the ovary of immature guinea pigs.

The degree of luteinization varied, however, and no factor other than the individuality of the animal could be found to explain the quantitative differences. The question is brought up of the threshold of reaction of tissues in any animal as a result of the normal biphasic hormone cycle which occurs even long before puberty. While this observation strengthens the belief in the luteinizing powers of the prehypophysis, it should stimulate further investigation of the guinea pig in the study of species difference and tissue responses.

Since the presentation of this paper attention has been called to the report of Selye, H., Collip, J. B., and Thomson, D. L., (PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 780) who treated 6 immature guinea pigs with 200 to 700 units of anterior pituitary-like hormone per day for 6 to 14 days, and produced luteal changes in the theca cells.

6848

Enteral Absorption of Insulin.

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Previous studies dealing with the enteral absorption of insulin have demonstrated the marked impermeability of the intestinal mucosa to this hormone.[1] It was considered of practical and theoretical interest to attempt to increase this degree of permeability by means of concomitantly administered drugs.

Normal dogs were prepared with Thierry loops about 12 cm. in length taken from about 50-60 cm. above the terminal ileum. Balloon and catheter arrangements similar to those described by Johnston[2] were used to insure retention of the introduced insulin mixture. This mixture was made up of Insulin-Lilly U-40 or U-80 and diluted with saline to 2½ or 3½ cc. before introduction. Blood samples were taken from the ear and sugar determined by the micro-method of Folin and Malmros.[3] (This method in our hands gives high absolute figures in the hypoglycemic range.) The dogs were ordinarily starved for 40 hours previous to the experiment. For purposes of brevity, the following hypoglycemic reaction, taken from the protocols is referred to as "typical hypoglycemia". Normal blood sugar 102 mg. %; 1½ hr. later 106. Insulin introduced. After 15 min., 94; 50 min., 65; 1½ hr., 65; 2 hr., 58; 2⅔ hr., 62; 3 hr., 79 mg. %.

With insulin in saline alone, about 35 units per kg. body weight represents the minimal dosage producing typical hypoglycemia.

One trial with an addition of hexyl resorcinol and toluene failed to show favorable influence on absorption. One trial with 0.3% saponin and 5% lactic acid produced typical hypoglycemia with ½ the usual amount of insulin.*

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[1] See references by Bollmann, J. L., and Mann, F. C., *Am. J. Med. Sci.*, 1932, **183**, 23, and Harned, B. K., and Nash, Jr., T. P., *J. Biol. Chem.*, 1932, **97**, 443.

[2] Johnston, C. G., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 193.

[3] Folin, O., *J. Biol. Chem.*, 1928, **77**, 421. Folin, O., and Malmros, H., *ibid.*, 1929, **83**, 115.

* The use of surface active agents, such as saponin, has been claimed by Lasch and Brugel[4] to produce hypoglycemia when administered orally with insulin. In 4 trials with rabbits we were unable to obtain such effects and are, accordingly, in agreement with the critical observations of Elzas,[5] Samek,[6] and Dingemanse and Laqueur.[7]

Several trials of insulin in a 20% alcohol solution produced typical hypoglycemia with dosages ½ to ⅓ those producing the same effect in saline alone. Typical hypoglycemia was produced with dosages as low as 10 units per kg., but the usual requirement is higher. The systemic effect of similar amounts of alcohol alone was demonstrated to be without influence.

The addition of lactic acid (1½ to 5%) to these mixtures does not decrease absorption and occasionally increases the effect.

Alcohol-lactic acid mixtures with insulin were introduced by catheter into one dog with a fistula anastomosed to communicate with the small gut about 50 cm. above the terminal ileum. Profound hypoglycemia leading to coma was produced with dosages of 32 and 40 units per kg. This dog, however, was in relatively poor shape and died 3 weeks after the last of these trials with partial obstruction from operatively produced adhesions.

Throughout the 35 trials in 6 dogs, the absorption effects were reasonably consistent with each individual dog although there was substantial variation between the different dogs.

We wish to thank Dr. K. K. Chen of the Lilly Research Laboratories for kindly supplying most of the insulin and to thank Dr. I. M. Gage for the operative preparation of one of the dogs.

6849

Roentgenologic Visualization of Lymph Nodes and Vessels by Intrapericardial Injection of Thorium Dioxide.*

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During previous experimental work on laboratory animals[1] it was noted that in the intracardiac injection of thorotrast, some of the material being injected also into the pericardium, roentgenograms

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[4] **Lasch and Brugel, *Arch. Exp. Path. Pharm.*, 1926, 120, 144.**

[5] **Elzas, *Nederland. Tijdschr. Geneeskunde*, 1926, 70, II, 1650.**

[6] **Samek, *Z. ges. Exp. Med.*, 1928, 62, 707.**

[7] Dingemanse and Laqueur, *Arch. Exp. Path. Pharm.*, 1927, **126**, 11.

* **Aided by a grant from the General Baking Company.**

[1] Ané, J. N., and Menville, L. J., *Am. J. Roentgenol. and Rad. Ther.*, 1932, **28**, 784.

made 24 hours after injection showed visualized mediastinal and diaphragmatic lymph glands and vessels.

The distribution of the lymphatic vessels of the heart and pericardium and the routes followed by carbon and carmine particles from these structures have been reported by many workers since the first account of Rudbeck. It is generally agreed that there are 2 main lymphatic trunks which convey lymph from the heart and pericardium. These 2 trunks, formed by major collecting channels at the origin of the coronary vessels, are designated by Kampmeier[2] as the "right" and "left" trunks, while Shore[3] considers them as the "anterior" and "posterior" trunks. While variations occur, it is believed that the right trunk generally passes anterior to the ascending aorta to enter the anterior mediastinal gland, while the left trunk, passing upward between the pulmonary artery and the left atrium, drains into the tracheo-bronchial lymph glands.

In the experiments here reported, several laboratory animals were used. Previous to injection with thorotrast, control films were made of these animals to determine the presence of any abnormality which otherwise might prove confusing on films made after injection. An attempt was made to inject the thorotrast into the pericardial cavity only, and films made immediately after injection demonstrated the presence of the opaque material within the pericardial cavity, surrounding the cardiac shadow.

Films made 24 hours after the intrapericardial injection of thorotrast demonstrated that the greater proportion of the material had been absorbed and distributed throughout the mediastinal, tracheobronchial, and diaphragmatic lymphatic glands and vessels, and a small amount remained in the pericardium. The liver and spleen were not visualized on these or subsequent films. This demonstrated that if any of the thorotrast entered the chambers of the heart, it was so slight that the organs of the reticulo-endothelial system were not visualized.

By the injection of particulate matter into the cardiac muscular wall, Kampmeier[2] confirmed the presence of deep lymphatic channels in the myocardium, which drained into the superficial or pericardial trunks. It is believed that in certain instances where we used the intrapericardial injections of thorium dioxide, it is possible that a small amount of the material was deposited in the myocardium, and if so, it drained through the deep lymphatics, entering the large trunks and eventually the lymphatic glands and vessels of the thorax. Further work on this subject is under way.

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[2] Kampmeier, Otto F., *Am. Heart J.*, 1928, **4**, 210.

[3] Shore, L. R., *J. Anat.*, 1928, **62**, 125, 291.

No deaths occurred nor were ill effects noted among the animals injected, all of which are in apparent good health 8 months after the injection.

These experiments serve as a radiographic demonstration of the lymphatic drainage of the pericardium, showing the routes through the anterior mediastinal and tracheo-bronchial nodes and vessels, then through the mediastinal lymphatic channels to the diaphragmatic lymphatic plexus.

Missouri Section.

St. Louis University School of Medicine, May 10, 1933.

6850

A Quantitative Procedure for Determining Normal Excretion of Prolan.

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Several methods have been developed for the preparation of active extracts of the gonad-stimulating substance of pregnancy urine. Although some of these procedures give good recoveries from pregnancy urine no one has reported a satisfactory procedure for the quantitative estimation of the small quantities of prolan that occur in normal urines. The excretion of prolan in conditions unassociated with pregnancy has been determined exclusively with untreated urine or by means of Zondek's[1] alcohol precipitation method which gives only a 5-fold concentration. Since alcohol causes an appreciable loss of activity (Wiesner and Marshall,[2] Katzman and Doisy,[3]) this procedure is not suitable for use in normal excretion of the anterior pituitary-like substance.

In attempting to purify pregnancy urine extracts by means of various protein precipitants we observed that precipitation with tungstic acid invariably gave remarkably complete recovery. Wiesner and Marshall[2] and Zondek, Scheibler and Krabbe[4] found that phosphotungstic acid quantitatively precipitates the active material of pregnancy urine but they have not reported studies on the recovery of the small amounts of prolan which occur in normal urine. We have not used phosphotungstic acid but it is evident from Table I that our tungstic acid method gives a surprisingly good recovery of minute quantities.

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[1] Zondek, B., *Klin. Woch.*, 1930a, **9**, 1209.

[2] Wiesner, B. P., and Marshall, P. G., *Quart. J. Exp. Physiol.*, 1931, **21**, 147.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

[4] Zondek, B., Scheibler, H., and Krabbe, W., *Biochem. Z.*, 1933, **258**, 102.

TABLE I.
Recovery of Prolan by Tungstic Acid Precipitation.

Volume of boiled male urine cc.	Prolan added	Prolan recovered	Recovery %	Remarks
2000*	45 R.U.	53 R.U.	118	
2000	45 "	47 "	105	
1000	13 "	>11 "	>85	not boiled
1000	17 "	18.7 "	110	" "
500A*	5 "	5 "	100	
500*	5 "	>4.2 "	>84	
500*	5 "	2.7 "	52	0.001% casein
500*	5 "	<2 "	<40	no protein added
400A*	4 "	>2.4 "	>60	
400A	4 "	4.2 "	105	
400*	4 "	4.2 "	105	
400	4 "	<3 "	<75	
1000	4.4 "	4.8 "	110	
1000	4.4 "	4.4 "	100	
500A*	5 "	5 "	100	H_2O used instead of urine
1000	4.4 M.U.	3.3 M.U.	75	"
1000	4.4 "	3.8 "	85	"

* not treated with $BaCl_2$.
A—albumen used instead of casein.

The procedure is as follows: A 24-hour urine sample to which has been added 10 cc. of 10% sodium tungstate and 10 cc. of 0.5% casein is made faintly acid to congo red with dilute H_2SO_4. The precipitate is collected by centrifugation, washed with acetone, freed from acetone by reduced pressure, and dissolved by adding dilute NaOH until the mixture is faintly alkaline to phenolphthalein. $BaCl_2$ and $Ba(OH)_2$ are then added in the proportion necessary to prevent the solution from becoming acid, until precipitation is complete. This precipitate is removed by centrifugation, and the excess barium is precipitated by Na_2SO_4, the $BaSO_4$ removed and the solution neutralized with dilute acetic acid. In this manner the original sample is concentrated to approximately 10 cc., representing a 100-fold concentration and permitting the determination of 3 mouse units per 24-hour sample. Few preparations are toxic if precipitation with barium is complete.

Table I shows that added amounts of prolan as small as 4.4 mouse units per liter can be recovered by this procedure. Addition of casein appears necessary since in the 2 cases where very little (0.001%) or none was added the recovery was not satisfactory.

The results from normal urines obtained by the tungstic acid method are shown in Table II. The presence of this factor in normal male urine has not been reported previously. Schörcher[5] did not find prolan in the urine of children and young people between the ages of 7 days and 20 years while Neumann and Peter[6] reported that it occurred occasionally between the ages of 3 months and 10 years. Our own data indicate that between the ages of 4 years and puberty little or no prolan is found in the urine, but that at puberty an increased excretion occurs.

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[5] Schörcher, F., and Zondek, B., *Klin. Woch.*, 1931, **10**, 2221.

TABLE II.
Prolan in Normal Urine.

No. subjects	Subject	Min. excretion per 24 hr.	Max. excretion per 24 hr.	Aver. excretion
4	Adult male	<4 M.U.	>19 M.U.	8 M.U.
4	Children before puberty			<2.8-4.7 M.U.
2	Children after puberty	14 M.U.	28 M.U.	21 M.U
7	Menopause	9 M.U.	26 M.U.	18 M.U.
4	Adult female	<3 M.U.	16 M.U.	10 M.U.

Several investigators have reported the presence of small amounts of prolan in the urine of normal women. Zondek,[5] working with mixed samples, found that a maximum excretion is reached just before and that a fall occurs immediately after menstruation. Frank, Goldberger, and Spielman[7] and Frank[8] have shown that the greatest concentration in the blood occurs in the early part of the cycle. Our own results on the daily excretion of 4 subjects show that 2 maxima occur, one during menstruation and the other about the time of ovulation, during which time the daily excretion rises to from 12 to 16 M.U. Between these peaks the values fall to 3 M.U. or less. Following the menopause we have found somewhat larger quantities (Zondek,[9] Österreicher[10]).

The earlier work of Frank and Goldberger[11] has led to an appreciation of the possibilities of the hormone analyses of blood and urine. We hope that a more thorough and systematic use of the method described in this paper will supply us with adequate data regarding normal physiological variations. Then perhaps the process may even be of aid to the clinician in diagnosis and treatment. In this connection, we call attention to the investigations of Fluhman,[12] Kurzrok,[13] McCullagh[14] and others.

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[6] Neumann, H. O., and Peter, F., *Z. f. Kinderh.*, 1931, **52**, 24.

[7] Frank, R. T., Goldberger, M. A., and Spielman, F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 999.

[8] Frank, R. T., *J. Am. Med. Assn.*, 1931, **97**, 1852.

[9] Zondek, B., *Klin. Woch.*, 1930, **9**, 393.

[10] Österreicher, W., *Klin. Woch.*, 1932, **11**, 813.

[11] Frank, R. T., and Goldberger, M. A., *J. Am. Med. Assn.*, 1926, **86**, 1686.

[12] Fluhman, C. F., *J. Am. Med. Assn.*, 1929, **93**, 672.

The tungstic acid process offers still other possibilities which have been partially explored. By certain modifications we have made preparations which show thyreotropic activity in the guinea pig and still other extracts which produce hyperglycemia in the rabbit. Kallikrein is likewise present in the crude extracts. Certain preliminary work indicates that the application of the process to pregnancy urine will greatly simplify the preparation of prolan.

6851

Effect of Tungstic Acid Extract of Normal Human Urine Upon Ovary of Rodents.

W. D. COLLIER AND N. J. WADE.

From the Departments of Pathology and Biology, St. Louis University School of Medicine.

This is a histological report on the study of the ovaries of 20 rats and 24 mice which had been injected by Drs. Katzman and Doisy with their tungstic acid extract of normal human urine.

All of the animals were injected on the twenty-first day of life and upon the 2 following days with 1 cc. of extract in the case of the mice and 2 cc. in the case of the rats. The mice were killed on the twenty-fourth or twenty-fifth day of life and the rats on the twenty-eighth or twenty-ninth day. Six rats and 8 mice were injected with the extract from the urine of adult males, 4 rats and 6 mice with the extract from the urine of normal females, 2 rats and 2 mice with the urine extract of pre-pubertal boys, one rat with the urine extract from a 16-year-old boy, one rat and 2 mice with the urine extract of an elderly female past the menopause, 6 rats and 6 mice with the urine extract from female castrates receiving theelin therapy.

The common qualitative effects are: inhibition of follicular growth in most cases, marked conversion of granulosa and theca of small follicles into interstitial gland substance, cystic degeneration of many of the larger follicles, luteinization of some large follicles with the formation of pseudo-corpora suggesting those of ovulation, lutein-

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[13] Kurzrok, R., *Endocrinol.*, 1932, **16**, 361.

[14] McCullagh, E. P., McCullagh, D. R., and Hicken, N. F., *Endocrinol.*, 1933, **17**, 49.

ization of granulosa and theca of many follicles, extensive atretic degeneration of follicles and congestion of vessels.

There is a wide quantitative variation in the degree of response produced by the extracts. The rats gave greater response than the mice but the latter were killed sooner after treatment. The extracts from the urine of female castrates receiving theelin therapy produced the greatest degree of change in the ovaries of the animals treated. The extracts from young males from 16 to 21 produced the next greatest degree of response. The extract from older males and adult females produced about the same degree of response but this degree was considerably less than that produced by the extracts from younger postpubertal males. The extracts from the urine of 2 prepubertal boys produced very slight changes in the ovaries of the treated animals and the extract from the post-menopause female produced only a slightly greater effect.

The production of luteinization of granulosa and theca and the production of great quantities of interstitial gland substance and the production of cystic atretic follicles strongly suggests a relationship between the action of this tungstic acid extract and that of prolan extracted by the benzoic acid method from urine of human pregnant females. We have recognized no definite follicle stimulating effect such as we have observed after the administration of prolan to rats and mice of the same age. We have not used comparable doses of the 2 extracts if the Katzman-Doisy unit can be accepted as the standard unit for both. The tungstic acid extract dose has been much smaller than the prolan doses that we have used so far.

6852

Nutritional Requirements of the Rabbit*

ALBERT G. HOGAN AND WALTER S. RITCHIE. (Introduced by P. A. Shaffer.)

From the Departments of Animal Husbandry and Agricultural Chemistry, University of Missouri.

There is abundant evidence[1] that the nutritional requirements of the rabbit are not the same as those of the rat. The possibility that the rabbit may require vitamin C has been considered by all workers in this field, but apparently none except Findlay[2] has found any reason to believe this requirement actually exists.

The first basal diet we used for the rabbit, No. 1002, had the following composition: Casein 20, starch 30, lard 13, cod liver oil 1, wheat germ oil 1, yeast 15, salts 5, cellulose 15. Ration 1535 was used later, and contained milk fat instead of lard, and cellophane instead of cellocotton. The longest survival period on Ration 1002 was approximately 7 weeks. Ration 1002 was then combined with various natural foodstuffs and observations were made on the growth rates and survival periods. The successful combinations are approximately as follows: one part Ration 1002 to: 1 wheat germ, 1 whole milk powder, 3.5 carrots, 6 whole milk, 5 canned tomatoes. In addition Ration 1535 was combined with 25 cc. daily of orange or lemon juice per rabbit. The response of the rabbits to the basal diets and to the various combinations is shown in Table I.

These feeding trials were conducted in an attempt to answer two questions: (1) Does the adequacy of a ration, satisfactory for the rabbit, depend in any marked degree on its composition as determined by the conventional feed analysis? (2) Do any of the substances which make the basal diet complete offer promise of yielding an extract that is equally effective? Our observations lead us to believe the first question can be answered in the negative, and the

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* Contribution from the Departments of Animal Husbandry and Agricultural Chemistry of the Missouri Agricultural Experiment Station. Journal Series No. 364.

[1] Nelson, V. E., Lamb, A. R., and Heller, V. G., *Am. J. Physiol.*, 1922, **59**, 335. Hogan, A. G., and Ritchie, W. S., *Mo. Agr. Exp. Sta. Bull.*, 1919-30, No. 300, 23. Goettsch, M., Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145. McCay, C. M., Madsen, L. L., and Maynard, L. A., *Proc. Am. Soc. Biol. Chem.*, 1933, lxviii.

[2] Findlay, G. M., *J. Path. Bact.*, 1921, **24**, 454.

TABLE I.
Observations on Growth of Rabbits

Ration	Number Observed Died	Survived	Initial Wt. gm.	Maximum Wt. gm.	Observed* days
1002	4	0	590-1100		
1002 + wheat germ	2	2	850-1100	2940-3000	131-177
1002 + whole milk powder	1	1	745-765	2730	169
1002 + carrots	2	3	530-1500	2530-3080	192-260
1002 + whole milk	2	2	730-1200	2920-3300	174-266
Whole milk	3	1	850-1150	2890	199
1002 + canned tomatoes	5	4	900-1150	2550-3800	177-347
1535	4	1	930-1090	2800	153
1535 + orange juice	2	2	675-860	3210-3300	201-225
1535 + lemon juice	1	1	740-930	2890	132

*Rabbits that died are not included.

second in the affirmative. The filtered juice of the orange or lemon (decitrated) were both effective supplements, though we regard orange juice as the most promising. Both sexes were represented in the survivors on this combination, so they were mated. The female bore a litter of 6 and reared 4. One rabbit grew to apparently normal maturity on Ration 1535 alone, so we regard it as more useful for our purpose than No. 1002.

Four guinea pigs have also been reared on the combination of Ration 1535 with 5 cc. daily per animal of one of the fruit juices, orange, lemon, or tomato. They did not grow rapidly and their hair lacks the sleek appearance of normally nourished animals, but they are still in fair condition after 131 days.

Our observations have not indicated any difference in the nutritional requirements of the guinea pig and rabbit except for vitamin C. Since we have reared several generations of rabbits on rations in which vitamin C could not be detected, we are convinced that, at most, provision of vitamin C is a minor problem in formulating a ration that is satisfactory for this animal. We are also convinced our early failures were not due to the provision of an unsuitable quantity of the indigestible component, though they may have been due to the physical state in which this component was supplied. We are now acting on the hypothesis that the failures are due to a deficiency of one or more vitamins, though we are unable to decide whether or not these vitamins are also required by the rat. We feel that our experience[3] with the chick lends some support to this position.

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[3] Hogan, A. G., and Boucher, R. V., *Proc. Am. Soc. Animal Production*, 1931, 45.

6853

The Relation of Serum Fractions to Serum Sickness in Rabbits.*

LLOYD JONES AND MOYER S. FLEISHER.

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

We have previously shown that injection of rabbits with from 5 to 10 cc. of horse serum per kilo of body weight, will cause the appearance of serum sickness in a large percentage of the animals. The reactions which we have considered as those of serum sickness are noted upon the rabbits' ears 4 to 8 days after injection.[1]

An attempt was made to determine whether or not the fractions euglobulin, pseudoglobulin and albumin of horse serum, might be or might contain the agent related to the causation of serum disease. Accordingly, these serum fractions[2] were injected into white rabbits in various dosages and with the results listed in Table I.

TABLE I.

Serum Protein Fraction	Mg. N. Inj. per kilo	No. Rabbits Injected	% Positive
Euglobulin	34-43	19	0
"	69-84	20	0
"	150	30	7
Pseudoglobulin	35	24	0
"	71-76	39	28
"	100	58	41
"	150	25	80
Albumin	43-54	18	0
"	86-108	14	0
"	150	20	0

The horse serum fractions used were from both normal and diphtheria toxin immune animals. In the present and in previous comparative studies, we have noted little or no difference between normal and immune sera, or between their respective fractions, in ability to cause serum sickness.

It is to be noted that a dose (per kilo of body weight) of 150 mg. of euglobulin nitrogen brought about serum sickness in 7% of 30 animals, while smaller doses of euglobulin were without effect in causing serum sickness. Pseudoglobulin was more active, with

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* This work was carried out under Grants No. 170 and No. 188 of the Therapeutic Research Committee of the American Medical Association.

[1] Fleisher, Moyer S., and Jones, Lloyd, *J. Exp. Med.*, 1931, **54**, 597.

[2] These fractions were kindly supplied by Sharpe and Dohme.

reference to the amounts injected, since a dosage of 150 mg. nitrogen per kilo brought about serum sickness in 80% of 25 animals. Smaller doses (*i. e.*, from 71 to 100 mg. nitrogen) of this fraction resulted in a decreased occurrence of serum sickness and since none of the animals receiving 35 mg. of nitrogen per kilo developed the disease, it may be inferred that under these conditions the threshold for development of serum sickness in the most susceptible animals lies at a dosage of between 35 and 71 mg. pseudoglobulin nitrogen per kilo. The albumin fraction, though administered in a dosage as large as 150 mg. nitrogen per kilo was without effect in causing serum sickness.

Under these conditions, it may be assumed that the inciting agent of serum sickness is encountered primarily in the pseudoglobulin fraction of horse serum.

6854

Preparation of Prolan, Theelin and Theelol from the Same Urine.

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From the Laboratory of Biological Chemistry, St. Louis University School of Medicine.

No doubt the possibility of preparing prolan, theelin and theelol from the same urine has occurred to many investigators interested in sex hormones. In this note we have given the adaptation of processes published from this laboratory which permits the preparation of the 3 substances. The combined methods which have been in constant use for about one year give a satisfactory yield of all 3 compounds.

The prolan is made by the benzoic acid process described by Katzman and Doisy.[1] The filtered urine is strongly acidified with hydrochloric acid and after a few days the supernatant liquid siphoned from the precipitate. The urine is extracted with butyl alcohol in the continuous extraction apparatus (Veler, Thayer and Doisy[2]). The solvent is distilled off, the residue leached with benzene and the benzene removed by distillation. The residue from 100 gallons of urine is dissolved in 1500 cc. of 80% ethyl alcohol containing 150 cc. of concentrated hydrochloric acid. The solution is hydrolyzed by boiling for 4 hours, the alcohol removed by distillation and solid NaOH added until the solution is alkaline to phenolphthalein. The solution is transferred to separatory funnels and extracted with butyl alcohol to which an equal volume of petroleum ether is added. The butyl alcohol-petroleum ether solution of theelin and theelol are then worked up according to the process described by Doisy and Thayer[3] beginning, however, at Step 3. We have found that the purification of theelol as the sodium salt (MacCorquodale, Thayer and Doisy[4]) to be quite advantageous.

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[1] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

[2] Veler, C. D., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.*, 1930, **87**, 357.

Though we have no quantitative data on the yield of theelin, the recovery seems to be quite satisfactory. The yield of theelol has ranged from 2 to 5 mg. per gallon of urine.

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[3] Doisy, E. A., and Thayer, S. A., *J. Biol. Chem.*, 1931, **91**, 641.

[4] MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A., *J. Biol. Chem.*, 1933, **99**, 327.

Minnesota Section.

University of Minnesota, May 24, 1933.

6855

Study of Iodine Number of Serum Fatty Acids in Infantile Eczema.

ARILD E. HANSEN. (Introduced by Irvine McQuarrie.)

From the Department of Pediatrics, University of Minnesota.

The observation that marked skin changes develop in rats suffering from the unsaturated fatty acid deficiency disease described by Burr and Burr[1] suggested the possibility that certain dermatological disorders of childhood, such as infantile eczema, might be dependent in part at least upon this type of dietary deficiency. A few preliminary observations on eczematous babies given liberal supplements of unsaturated fatty acids in their diets in addition to the routine treatment were sufficiently encouraging to suggest the desirability of careful study of the blood lipids in this disease. In order to determine whether any relationship might exist between the two clinical entities, similar blood studies were undertaken on eczematous infants and on rats suffering from the unsaturated fatty acid deficiency.

The present paper deals exclusively with the results in the cases of infantile eczema. The total iodine absorption of the serum was first determined on one or more occasions in 10 cases of eczema and in 16 normal infants of similar age. This was followed by determinations of the total fatty acids, cholesterol, total iodine absorption of the serum and the iodine number of the serum fatty acids in 5 normal and 6 eczematous infants.

All blood samples were collected between 12 and 16 hours after a meal. Bloor's methods[2] were used to determine the cholesterol and total fatty acids in the serum. The Rosenmund-Kuhnhenn[3] method as modified by Page, Pasternak and Burt[4] was used to determine the iodine absorption of the serum lipids.

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[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; 1930, **86**, 587.

[2] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[3] Rosenmund, K. W., and Kuhnhenn, W., *Z. f. unter. d. Nahr. d. Nahr. u. Genuss.*, 1923, **46**, 154.

The preliminary data on the total iodine absorption values of the sera are as follows: The average for 21 determinations on 10 infants with eczema was 383 mg. (range 280-487) iodine absorbed per 100 cc. serum. The corresponding average for 18 determinations on the 16 control infants was 539 mg. (range 460-711).

The results from the more complete study of the blood lipids are shown in Table I.

TABLE I.
Serum lipids and iodine numbers of serum fatty acids in infantile eczema and in normal infants, age 5 to 18 mos.

	I_2 absorbed	Cholesterol	Total fatty acids	Total lipids	I_2 No.
			Mg. per 100 cc. serum		
			Normal infants		
	514	213	361	574	104
	477	160	339	499	109
	711	232	411	643	136
	508	150	407	557	101
	386	142	292	434	100
	540	213	354	567	113
Aver.	526	185	361	546	111
			Children with infantile eczema		
	404	143	443	586	69
	416	118	317	435	104
	362	142	328	470	82
	417	159	340	499	91
	370	150	345	495	78
	307	148	261	409	80
	416	160	360	520	86
Aver.	385	147	342	488	84

The serum cholesterol and total fatty acids are slightly less in the eczema cases than in the control infants. The average iodine number of the serum fatty acids in the eczema group is 84 while in the control group the average iodine number is 111. This indicates that the serum fatty acids are less unsaturated in infantile eczema than in the control infants. Hansen and Burr[5] have shown that the serum fatty acids are less unsaturated in rats suffering from the unsaturated fatty acid deficiency disease. While this evidence does not preclude the possibility of a lipid-protein combination, if these data can be substantiated further, it appears that the unsaturated fatty acids must be given serious consideration as an etiological factor in infantile eczema. Further studies are now in progress to determine this point.

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[4] Page, H. H., Pasternak, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

[5] Hansen, Arild E., and Burr, George O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1201.

6856

Studies on Iodine Absorption of Serum in Rats Fed on Fat-free Diets.*

ARILD E. HANSEN AND GEORGE O. BURR.

From the Departments of Pediatrics and Botany, University of Minnesota.

In view of the apparent necessity for unsaturated fatty acids in the diet, it was thought that a preliminary comparison of the iodine absorption of the serum lipids of control rats with that of animals fed on fat-free diets might show significant differences.

The animals were chloroformed and the blood was collected directly from the heart into a test tube, pressure being made on the body to insure maximum collection. The serum was obtained by centrifuging. An alcohol-ether extract of the serum was prepared according to Bloor's method.[1] The iodine absorption was determined according to the method of Rosenmund-Kuhnhenn[2] as modified by Page, Pasternak and Burt.[3] Determinations were made directly on the alcohol-ether extract. Eight healthy control animals and 8 fed on a fat-free diet were used. The results obtained (Table I) are expressed in mg. iodine absorbed per 100 cc. serum.

The differences between the two groups are distinct. In fact, the groups are separated so that the highest of the "fat-free" animals does not reach the lowest of the controls. These results are especially significant in view of the fact that the controls are on a relatively low fat diet (McCollum's diet No. 1),[4] in which the fat is largely from milk. Therefore, the bodies of these animals probably have fats of as low iodine number as any animals on ordinary mixed diets.

This work is being extended to show whether the low iodine absorption of the alcohol-ether extract of the serum of the fat-starved rats is due to smaller amounts of fats or to a lower iodine

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* This work was supported by grants from the National Live Stock and Meat Board, the Medical Research Fund and General Research Fund of the University of Minnesota.

[1] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[2] Rosenmund, K. W., and Kuhnhenn, W., *Z. f. unter. d. Nahr. u. Genuss.*, 1923, **46**, 154.

[3] Page, H. H., Pasternak, L., and Burt, M. L., *Biochem. Z.*, 1930, **223**, 445.

[4] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, **33**, 63.

TABLE I.
Mg. iodine absorbed per 100 cc. serum of normal rats and of rats fed on a fat-free diet.

	Normal rats on stock diet* Iodine absorbed	Rats on a fat-free diet** Iodine absorbed
	501	330
	435	379
	670	395
	917	271
	646	296
	583	273
	530	277
	505	252
Aver.	598	309

*McCollum's diet No. 1.[4]
**550 B + vitamin supplement, Burr and Burr.[5]

number of the fats. The work of Bloor,[6] Maynard and McCay,[7] and Bender and Maynard[8] indicates large drops in the phosphatides, cholesterol and total lipids of the blood when low fat diets are fed. No iodine number of the serum fatty acids is given by these workers.

6857

Iodine Numbers of Serum Lipids in Rats Fed on Fat-Free Diets.*

ARILD E. HANSEN AND GEORGE O. BURR.

From the Departments of Pediatrics and Botany, University of Minnesota.

Our preliminary data[1] indicated that the serum from rats fed on fat-free diets absorbed less iodine than the serum from control animals. We have since attempted to determine whether the differences found could be due to smaller amounts of total lipids or to lipids having a lower iodine number.

The alcohol-ether extract of the serum was prepared in the usual manner.[2] The iodine absorption was determined on the alcohol-

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[5] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, **86**, 587.

[6] Bloor, W. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 701.

[7] Maynard, L. A., and McCay, C. M., Cornell University Agricultural Experiment Station, Bull. 543, September, 1932.

[8] Bender, R. C., and Maynard, L. A., *J. Dairy Sci.*, 1932, **15**, 1.

* This work was supported by grants from the National Live Stock and Meat Board, from the Medical Research Fund and General Research Fund of the University of Minnesota.

[1] Hansen, Arild E., and Burr, George O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1200.

[2] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

ether extract of the serum as previously described.[1] Bloor's method[2] was used to determine the total fatty acids and cholesterol.

The results are given in Table I.

TABLE I.
Serum lipids and iodine numbers of serum fatty acids of rats on a fat-free diet and of control rats.

			Normal control rats*		
	I_2 absorbed	Cholesterol	Total fatty acids	Total lipids	I_2 No.
			Mg. per 100 cc. serum		
	431	111	280	391	128
	428	116	271	387	129
	548	84	306	390	160
	308	74	209	283	124
	554	113	367	480	130
	478	118	292	410	137
	436	102	256	356	145
Aver.	455	102	283	385	136
			Rats on a fat-free diet**		
	282	93	200	293	110
	393	110	290	400	110
	328	67	231	298	122
	444	109	327	436	114
	530	106	437	543	105
	309	88	190	278	132
	423	140	283	423	117
	330	100	218	318	121
	262	84	212	287	108
	330	75	252	337	100
	308	98	211	308	111
	269	87	285	372	74
	267	78	239	317	90
	267	86	232	318	89
Aver.	339	87	258	352	107

*McCollum's diet No. 1.[3]
**550 B + vitamin supplement, Burr and Burr.[4]

In the previous study the animals were not starved before the specimens were obtained. In this series uniform starvation periods were adopted in order to eliminate abnormal values due to the post-absorptive state. The average values of the cholesterol and the total fatty acids are less in the experimental animals than in the control groups, which agrees with findings of Bloor,[5] Maynard and McCay,[6] and Bender and Maynard.[7] The iodine numbers indicate that the serum fatty acids of the rats fed on a fat-free diet are less unsaturated than those of the controls.

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[3] McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, **33**, 63.

[4] Burr, George O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; 1930, **86**, 587.

[5] Bloor, W. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 701.

[6] Maynard, L. A., and McCay, C. M., Cornell University Agricultural Experiment Station, Bull. 543, September, 1932.

[7] Bender, R. C., and Maynard, L. A., *J. Dairy Sci.*, 1932, **15**, 1.

Summary. (1) The cholesterol and total fatty acids of the serum in rats fed on a fat-free diet were found to be lower than in control animals. (2) The iodine number of the serum fatty acids from rats fed on fat-free diets indicates that the fatty acids are less unsaturated than those of controls.

6858

Survey to Determine the Incidence of Bang's Disease in Minnesota Cattle.*

C. P. FITCH AND C. R. DONHAM.

From the University of Minnesota, University Farm, St. Paul, Minn.

Estimates of the prevalence of Bang's disease (contagious abortion) have been based on the results of agglutination tests that are available from laboratories engaged in the control of this disease. Such data do not give a true picture because the serum samples are from herds that request service. Usually testing is carried out only in herds in which there is reason to suspect the presence of Bang's disease. Also, retests of negative herds (from which the disease has been eliminated) tend to invalidate such figures as an estimate of the prevalence of this disease.

Reliable data on the incidence of Bang's disease are needed, not alone for obvious economic reasons, but also to provide a basis of estimation of the dependability of the agglutination method as a control measure for this disease. It has been shown,[1] by adequate experiments, that the test-tube agglutination test is exceedingly accurate with serums having either no specific agglutinin content or high agglutinin content (titre 1:250 or above). It has also been shown, however, that serums having a low to medium agglutinin content (titre 1:25 to 1:100) frequently give variable agglutination

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* Some of the researches upon which this paper is based were made possible by a grant from the Bureau of Animal Industry of the United States Department of Agriculture. Published with the approval of the Director as Paper No. 1182 of the Journal Series of the Minnesota Agricultural Experiment Station.

[1] Fitch, C. P., Donham, C. R., Bishop, Lucille M., and Boyd, W. L., *Minn. Tech. Bull.*, 1930, 73.

titres in different laboratories and in duplicate tests in the same laboratory. Therefore, it is obvious that the dependability of the agglutination method as a control measure, is inversely proportional to the percentage of animals having serums with low to medium agglutinin content (suspicious diagnosis).

We are attempting to obtain a more reliable estimate of the incidence of Bang's disease based on the total cattle population. The Minnesota State Live Stock Sanitary Board is cooperating in this work. The township has been selected as a representative sample of an area. At least 85% of the herds in a township are included in the study. The test-tube agglutination test, along with the age and as complete breeding history of the individual animals as can be obtained, form the basis of diagnosis. The agglutination tests were conducted in accordance with the standard adopted by the United States Live Stock Sanitary Association,[2] except that a wider range of dilutions was employed, namely: 1:25, 1:50, 1:100, 1:250, 1:500, and 1:1000. All the bovine animals in the herd are included in the study.

It is anticipated that at least 6 different areas in widely scattered sections of the state will be included in the survey. These areas are being selected to represent all of the different types of the cattle industry found in Minnesota. The work has been completed in 2 areas, namely: Oakdale Township, Washington County, and Deer

TABLE I.
Results of test-tube agglutination test for Bang's disease of cattle in two Minnesota townships.

Diagnosis	Oakdale Township Washington County		Deer River Township Itasca County		Total of both Townships	
Animals	No.	%	No.	%	No.	%
Negative	1936	86.0	875	93.9	2811	88.3
Suspicious	160	7.1	28	3.0	188	5.9
Positive	155	6.9	29	3.1	184	5.8
Total animals	2251	100.0	932	100.0	3183	100.0
Herds						
Negative	56	52.3	64	86.5	120	66.3
Suspicious*	16	15.0	7	9.5	23	12.7
Positive**	35	32.7	3	4.0	38	21.0
Total herds	107	100.0	74	100.0	181	100.0

*Herds with one or more suspicious animals but no positive animals.
**Herds with one or more positive animals.

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[2] Report of 1931 Committee on Bang's Disease (Part I) of the United States Live Stock Sanitary Assn. *J. A. V. M. A.*, 1932, LXXX, n. s. 33 (3), 323.

River Township, Itasca County. The results are given in the accompanying table.

Results. Out of a total of 3,183 animals tested, 88.3% were negative, 5.9% were suspicious and 5.8% were positive. There were 181 herds of cattle with an average of 17.6 animals per herd. Of these herds, 66.3% were entirely negative, 12.7% had one or more suspicious animals but no positive animals, and 21.0% had one or more positive animals. Only one herd showed 100% of suspicious and positive animals. There were 13 animals in this herd, all more than 3 months of age.

These figures indicate that Bang's disease is much less prevalent, both in percentages of animals and herds affected, than has previously been estimated. Also, that Bang's disease does not usually affect all of the animals in a herd.

6859

Presence of Bact. Abortus Bang in Raw Milk, Butter and Ice Cream.*

C. P. FITCH AND LUCILLE M. BISHOP.

From the University of Minnesota, University Farm, St. Paul, Minnesota.

The milk ordinances of two municipalities were examined. One municipality required all milk sold as "raw" to come from cattle negative to the agglutination test for Bang's disease, while the other ordinance had no such requirement. Eighteen quart samples of milk were collected from the commercial distributors of each city, representing 17 dairies in one and 18 dairies in the other. Three to 5 cc. of gravity cream from each quart was inoculated intraperitoneally into 2 guinea pigs. The pigs surviving the inoculations were autopsied 4 weeks later. The spleen of each was cultured for *Bact. abortus* and the blood tested for agglutinins for this organism.

Bact. abortus was not isolated from any of the quart samples of milk from the 18 dairies in the city, the ordinance of which required raw milk to come from cattle tested for Bang's disease. Two guinea pigs, representing one dairy, died as a result of the inoculation before the end of the 4-week incubation period, so that satisfactory results could not be obtained. Agglutinins for *Bact. abortus* were not demonstrated in any of the milk from this city. Guinea pig inoculations of the milk from dairies supplying milk to the other city were as follows: Six pairs of pigs inoculated with milk from 6 dairies, died in from 1 to 10 days after injection and satisfactory incubation periods for *Bact. abortus* were not obtained. Agglutinins for *Bact. abortus* were demonstrated in the milk from 2 of the above dairies in the 1:50 dilution. Therefore, milk containing these agglutinins was being used to make up these milk supplies. Usually milk from a single udder with this amount of agglutinin does not contain *Bact. abortus;* however, these agglutinins were no doubt diluted many times with milk from negative animals and this low agglutination of the mixed milk probably indicated infection in the herd. Milk from the remaining 12 samples gave the following results: *Bact. abortus* was not isolated from 6 samples and was isolated from 6. The latter samples represented 5 dairies as duplicate samples were obtained from one dairy.

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The fact that *Bact. abortus* may be found in milk has been known since Smith and Fabyan[1] and Schroeder and Cotton[2] described the lesions produced in guinea pigs by the injection of milk containing this organism. Our preliminary experiments and work of other investigators (Huddleson,[3] Carpenter and Baker[4]) indicate that gravity cream is the best portion to use for isolation of *Bact. abortus.* For this reason products containing large quantities of raw cream would also be expected to contain this organism. Carpenter and Boak[5] collected 17 samples of market butter but did not isolate *Bact. abortus* from any of them. They found, however, if butter was artificially inoculated with this organism that it would survive from 32 to 142 days at 8°C. Van der Hoeden[6] examined 20 samples of sour cream butter made from naturally infected milk but did not isolate *Bact. abortus.* He was of the opinion, as was Carpenter and Boak that this organism was destroyed as the acidity approached a pH of 5. Lerche[7] isolated *Bact. abortus* 5 times from

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[1] Smith, T., and Fabyan, M., *Centralbl. f. Bakteriol.*, 1912, **61**, 549.

[2] Schroeder, E. C., and Cotton, W. E., *Rep. Bureau Animal Industry, U. S. Dept. Ag.*, 1911, **28**, 139.

[3] Huddleson, F., Halsey, D. E., and Torrey, J. P., *J. Inf. Dis.*, 1927, **40**, 352.

[4] Carpenter, C. M., and Baker, D. W., *Cornell Vet.*, 1927, **17**, 236.

[5] Carpenter, C. M., and Boak, R., *Am. J. Pub. Health*, 1928, **18**, 743.

[6] Van der Hoeden, J., *Tijdschr. v. Diergeneesk.*, 1929, **56**, 217. Ref. *Berl. Tierärztl. Woschr.*, 1930, **46**, 86.

[7] Lerche, *Z. f. Infektionskr.*, 1930, **38**, 253.

14 samples of butter made from infected cream and once from 11 samples of buttermilk. He was unable to isolate *Bact. abortus* from 12 samples of cottage cheese made from infected milk.

In our own experiments cream was collected by gravity separation from milk of cattle known to be shedding *Bact. abortus.* The gravity cream was not pasteurized and while still sweet was made into butter; however, commercially prepared butter is commonly made from pasteurized cream. One-half of the butter made from the cream was salted (3%) and one-half was not. The buttermilk was also collected. Guinea pigs were inoculated with these products shortly after they were made. Three such experiments were performed. *Bact. abortus* was isolated from the buttermilk, the salted and the unsalted butter. Two experiments have now been completed in which *Bact. abortus* was recovered from ice cream prepared from cream known to be naturally infected. Experiments are in progress to determine how long *Bact. abortus* will survive in butter and ice cream.

6860

Isolation of Mesobiliviolin from Human Feces. Its Origin and Nature.

C. J. WATSON. (Introduced by George Fahr.)

From the Department of Medicine, University Hospital, Minneapolis.

Coincident with the isolation of crystalline stercobilin,[1, 2] a substance resembling mesobiliviolin as first described by Fischer and Niemann[3] was regularly found in human feces.[2] Mesobiliviolin, as described by Fischer and Niemann,[3] exhibited absorption in the region of 600 and 500 mm. and showed green fluorescence with Zn acetate, the solution of the zinc salt having absorption at 626, 573, and 500. The similar substance found in feces had very similar characteristics,[2] and was consequently given the name kopromesobiliviolin.

It has now been determined that this substance still contained stercobilin as an impurity which accounted for the absorption in the region of 500 mm. as well as the green fluorescence of the zinc salt.

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[1] Watson, C. J., *H. S. Z.*, 1932, **204**, 57.

[2] Watson, C. J., *H. S. Z.*, 1932, **208**, 101.

[3] Fischer, H., and Niemann, *H. S. Z.*, 1924, **137**, 292.

Their complete separation has now been effected by virtue of the somewhat greater solubility of mesobiliviolin in ether. After this separation, the violet pigment obtained possesses no "urobilin" absorption. Its hydrochloride has a more bluish violet color with 2 closely adjacent absorption bands, the narrower from 608-598 (max. 604) and the broader 594-550 (max. 575). The free substance has one broad band 587-570 (max. 578-582). On treatment with alcoholic zinc acetate the color of the solution becomes a light blue and a beautiful intense red fluorescence is exhibited. This solution has the following absorption: 625-627 (well defined, narrow), 583, max. (faint, broader).

The most highly purified substance was obtained in the following way: (1) repeated acetic ether extraction of the stool; (2) removal of the ether by vacuum distillation; (3) precipitation of fatty acids and koprosterin by dilution of the remaining acetic acid solution with a large volume of 1% HCl; (4) repeated ether extraction after making the aqueous filtrate congo negative with sodium acetate. After washing the ether with water until the latter no longer showed absorption at 500 mm., the following fractionation was carried out:

$$\text{Ether} - 1\%\ \text{HCl} - \text{CHCl}_3 - \text{soda}\ \frac{\text{acidification}}{\text{with acetic}} - \text{ether (washed with } \text{H}_2\text{O}) -$$
$$1\%\ \text{HCl} - \text{CHCl}_3 - \text{soda}\ \frac{\text{acidification}}{\text{with acetic}}\ \text{CHCl}_3.$$

After concentration *in vacuo,* the mesobiliviolin was precipitated by pouring the solution into a large volume of petroleum ether. This precipitation was repeated, and the precipitate then repeatedly extracted with dry ether, in which the substance is only a little soluble. After concentration *in vacuo,* and slow evaporation, a separation of what were apparently very small prisms took place. Because of their deep color and minute size it was impossible to positively identify the material as crystalline. The substance is not particularly stabile. Its solutions are definitely sensitive to light, and to evaporation in the presence of air. Attempts to obtain a more definitely crystalline material have not yet been successful. A material of the same appearance as that obtained out of ether may be had by adding boiling isopropyl ether to a hot chloroform solution, and further concentrating.

This substance decomposes below its melting point, exhibiting shrinking at 178-180°C., swelling and decomposition with evolution of gas at 240-245°C.

For purposes of comparison the same procedure as outlined above was applied to mesobiliviolin as obtained by the iron chloride and heat oxidation of mesobilirubinogen (urobilinogen) according to the method of Fischer and Niemann.[3] It was found that the urobilin or stercobilin absorption band could in the same way as above be completely removed from the violet pigment, which thereafter exhibited the same absorption as that described for the mesobiliviolin out of feces, as well as strong red fluorescence with the zinc acetate, the zinc salt also having identical absorption.

Mesobiliviolin obtained by the above method *in vitro* from mesobilirubinogen has no definite melting point, exhibits shrinking from 135 to 168° at about which temperature some softening occurs without definite melting; at 230° swelling and apparent decomposition takes place. It is hoped that sufficient kopro-mesobiliviolin will soon be available to permit amalgam reduction with a view to obtaining a crystalline leukobase such as Fischer and Niemann prepared from mesobiliviolin (mesobiliviolinogen).

In the earlier literature there was occasional mention of the occurrence of bilicyanin or cholecyanin in human feces and urine.[4, 5] Fischer and Adler[6] found that certain of the blue oxidation products of bilirubin exhibit red fluorescence with zinc acetate. For this reason bilirubin was oxidized after the method of Heynsius and Campbell,[4] who originally described bilicyanin, and the blue and violet stages were compared spectroscopically with mesobiliviolin from feces and that obtained *in vitro* from mesobilirubinogen. Although the former in some instances exhibited red fluorescence with zinc acetate they were not identical spectroscopically with either of the mesobiliviolins.

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[4] Heynsius and Campbell, *Pflügers Arch.*, 1871, **4**, 520.

[5] Mueller, F., *Virch. Arch.*, 1893, **131**, 106.

[6] Fischer, H., and Adler, E., *H. S. Z.*, 1932, **206**, 187.

6861

Isolation of Crystalline Urobilin from Human Urine.

C. J. WATSON. (Introduced by George Fahr.)

From the Department of Medicine, University Hospital, Minneapolis.

Following the isolation of crystalline stercobilin from feces[1, 2] attempts had been made to isolate the corresponding substance, urobilin, from urine. These were unsuccessful. The amalgam reduction of crystalline stercobilin did not yield mesobilirubinogen.[2] Neither did its oxidation with nitric acid yield methyl-ethyl-malein imid which under the same conditions and with the same amounts of substance is easily obtained from mesobilirubinogen. Urobilinogen from urine had already been shown to be identical with mesobilirubinogen, the latter[3] obtained by the amalgam reduction of bilirubin. These facts suggested that stercobilin from feces and urobilin from urine were probably not identical, although very similar. Newer evidence, however, indicates a different explanation of the above findings.

The complete separation of mesobiliviolin and stercobilin, as described in this issue, suggested the application of this principle to extracts of urines rich in urobilin. This has been done with the result that crystalline urobilin was readily obtained. Since both stercobilin and urobilin do not melt sharply, decomposing between 110-130°, other means of identification are necessary. Crystallographic studies as well as measurements of the refractive indices of the two substances are under way. However, their other characteristics including solubilities, absorption spectra, Ehrlich reaction after amalgam reduction, and zinc salts are apparently identical. Preliminary studies of the crystals have shown that both exhibit parallel extinction. Comparative elementary analyses are being carried out.

The crystalline substance was obtained out of several liters of urine rich in urobilin by the following procedure: The urine was strongly acidified with acetic, and shaken out repeatedly with small amounts of chloroform until both fractions no longer showed green fluorescence with zinc acetate. The emulsified portion was shaken with talc and filtered with suction. The chloroform was repeatedly

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[1] Watson, C. J., *H. S. Z.*, 1932, **204**, 57.

[2] Watson, C. J., *H. S. Z.*, 1932, **208**, 101.

[3] Fischer, H., and Meyer-Betz, *H. S. Z.*, 1911, **75**, 232.

extracted with distilled water. A considerable portion of the urobilin went into the water, while mesobiliviolin and other impurities remained in the chloroform. The watery solution was made acid with HCl, and the urobilin hydrochloride returned to chloroform. It was returned to 25 HCl, which was diluted to 8% and shaken out again with chloroform. The latter was dried superficially over sodium sulfate, concentrated to a small volume and poured into a large volume of petroleum ether. The precipitated urobilin hydrochloride was redissolved in chloroform and again precipitated with petroleum ether. It was then dissolved in hot chloroform and crystallized on cooling. It was readily recrystallized in the same way.

The urine employed was from a patient with cardiac failure, large liver and ascites. It did not contain bilirubin. The theoretical yield in the above instance was 112 mg. The actual yield of recrystallized urobilin hydrochloride was 15 mg.

Employing the soda-ether-1% HCl-$CHCl_3$ fractionation as for mesobiliviolin and stercobilin, mesobiliviolin, which exhibited the typical absorption spectrum as well as red fluorescence of the zinc salt with absorption at 627 and 583 mm., was obtained from the chloroform fraction of the above after the aqueous extraction. Mesobiliviolin has been observed in the extracts of other urobilin containing urines as well.

Thus it appears that the leukobase of bilirubin (urobilinogen or mesobilirubinogen), which is formed in the bowel, yields at least 2 oxidation products, occurring in the feces and urine, *i. e.,* urobilin or stercobilin and mesobiliviolin.

Preliminary attempts have been made to isolate crystalline urobilin following the *in vitro* oxidation of mesobilirubinogen. A urobilin-like substance regularly occurs along with mesobiliviolin. Employing the above mentioned fractionation, a small crystallization on one occasion took place. The crystals were apparently identical with those of urobilin or stercobilin, and came out of chloroform in the same way. As yet, however, the amount obtained is too small to permit any definite conclusion. This will be repeated using larger amounts of mesobilirubinogen.

6862

Lowered Colloid Osmotic Pressure Leads to Water and Salt Retention and Edema Formation.

GEORGE FAHR AND ARTHUR KERKHOF.

From the Department of Medicine, University of Minnesota, and the Minneapolis General Hospital.

Eight hundred cc. of blood were removed by puncture of the right ventricle from a dog weighing 13 kilos. The citrated blood was centrifuged and the supernatant plasma pipetted off. The corpuscles were suspended in a quantity of Ringer's solution equal to the plasma pipetted off, again centrifuged and the supernatant Ringer's solution pipetted off. The corpuscles were now suspended in Ringer's solution containing 0.1% glucose in quantity sufficient to bring up the total volume to 800 cc. This was injected into the jugular vein. 200 cc. of Ringer's solution were also injected into the jugular vein. This procedure, bleeding, removal of plasma, suspension of corpuscles in Ringer's solution and reinjection into the jugular vein, was done 5 times in 18 hours. The plasma proteins were 1.7% at the time of the third removal of blood from the dog's ventricle. The plasma proteins were 2.2% and the osmotic pressure as measured in the osmometer was 5.0 mm. Hg. at the beginning of the fifth removal of blood plasma.

The dog received 500 cc. of physiological salt solution by stomach tube at 5 different times, 2500 cc. in all. In addition he drank voluntarily 1260 cc. of water. The dog received a total of 4760 cc. of water and 31.5 gm. of salt above what his body contained at the start of the experiment. The dog put out 950 cc. of urine containing 9.3 gm. of salt in 26 hours, 3800 cc. of water and 22.5 gm. of salt were retained. The dog gained 3300 gm. in weight in 22 hours. The dog showed pitting edema and ascites at the end of 26 hours when he was sacrificed. There were 500 cc. of ascitic fluid in the abdomen and there was edema of the subcutaneous tissues of the legs at autopsy examination. Examination of the kidneys revealed normal kidneys.

The same procedure was applied to another dog of 9 kilos excepting that there were only 4 removals of blood and only 700 cc. of blood were removed each time. After removing the supernatant plasma and washing the corpuscles in Ringer, 350 cc. of Ringer's solution containing 0.1% glucose and enough gum acacia to make up a 6% solution of gum acacia[1, 2] was added to the corpuscle volume. This mixture was injected back through the jugular vein together with an extra 200 cc. of Ringer's solution. The plasma proteins were 2.1% and the osmotic pressure of the plasma colloids were 20 mm. Hg. at the beginning of the second bleeding. The plasma proteins were 1.0% at the beginning of the fourth bleeding. The plasma proteins were 0.9% and the colloid osmotic pressure was 15 mm. Hg. 22 hours after beginning the experiment. The dog had received 3300 cc. of water and 20.8 gm. of NaCl within the 22 hours. 800 cc. of this water and 6.4 gm. of salt were injected intravenously. He put out 2600 cc. of urine in this time and 20.1 gm. of salt. His weight increased only 450 gm. in 22 hours. At autopsy there was no ascites nor signs of edema in the subcutaneous tissues or muscles excepting around the jugular veins in the neck where the gum acacia solution had been injected intravenously. Local edema about the intravenous injection site is a common finding in these experiments when the colloid osmotic pressure is lowered to 16 mm. Hg. or less, even where there is no marked retention of water and no generalized edema.

A third dog weighing 8 kilos received 2500 cc. of water and 20.8 gm. of salt, in the same manner except that no blood was removed, no plasma protein removed and no acacia given. 800 cc. of this water and 6.4 gm. of salt were injected intravenously. This dog urinated 2400 cc. containing 19.9 gm. of salt. The dog lost 500 gm. in weight in 22 hours.

These experiments prove conclusively that lowering the colloid osmotic pressure to ⅓ its normal value at the same time that large quantities of water and salt are given leads to water and salt retention and edema. And they also prove that it is the osmotic pressure and not the presence of protein that is responsible for the maintenance of normal water and salt balance in the body.

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[1] Bayliss, W. M., *Proc. Royal Soc., London,* 1916, **89**, 380.

[2] Hartman, Alexis, *et al., J. Am. Med. Assn.,* 1933, **100**, 251.

Iowa Section.

State University of Iowa, May 13, 1933.

6863

Presence of SH-Compounds in Non-Developing Embryos.

TITUS C. EVANS. (Introduced by J. H. Bodine.)

From the Zoology Laboratories, State University of Iowa.

Hammett[1] points out that the —SH group is the essential chemical stimulus for cell division. He also concludes[2] that the greater the natural cell division activity the greater the degree of reaction to the chemical groups. Coldwater[3] regards the nitroprusside reaction as indicating the presence of protein-fixed —SH groups, and finds a high concentration of this sulphydryl in areas of regenerating tissues undergoing proliferative growth. Thompson and Voegtlin,[4] Murray,[5] and Yaoi[6] have found the glutathione content of embryos of the chick and rat to be at a maximum in early development and to decrease with age.

One might infer from the above that if embryos were tested for the —SH groups at periods of marked cellular activity a much stronger reaction would be obtained than if they were tested at periods of relative inactivity. The embryo of the grasshopper, *Melanoplus differentialis*, is favorable material for this test as it can be obtained in large numbers, is easily dissected out, and is so small that it is quickly saturated with the sodium nitroprusside. Its development may be divided into 3 definite periods. First, a period of rapid embryonic growth for 21 days (at 25° C.) which is followed by a period of apparent inactivity lasting from a few days to several months. This period (diapause) is marked by the lack of any movement, absence of mitosis (Slifer[7]) and very low rates of respiratory metabolism (Bodine[8]). The third period begins with the resumption of mitotic activity followed by blastokinesis. After the embryo has revolved, it grows rapidly and engulfs the remaining yolk. The third phase marks the completion of differentiation and embryonic organogenesis.

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[1] Hammett, P. S., *Protoplasma*, 1929, **7**, 297.

[2] Hammett, F. S., and Hammett, D. W., *Protoplasma*, 1932, **16**, 253.

[3] Coldwater, K. B., *J. Exp. Zool.*, 1933, **65**, 43.

[4] Thompson, J. W., and Voegtlin, C., *J. Biol. Chem.*, 1926, **70**, 793.

[5] Murray, H. A., *J. Gen. Physiol.*, 1926, **51**, 613.

[6] Yaoi, H., *Jap. J. Exp. Med.*, 1928, **7**, 135.

This preliminary report on the glutathione reaction is based upon observations of 13 embryos in early development, 70 embryos in diapause, and 30 in various stages of later development. The nitroprusside test for —SH groups was used and the color lasted from 18 to 90 seconds. All embryos (even those in diapause) gave a definite positive color reaction. The stain was apparently more intense and lasting where the tissues were thicker. Yolk did not stain and embryonic membranes colored only momentarily.

It is significant that embryos in all stages of diapause showed an intense color reaction indicating the presence of the protein bound —SH groups. It is possible that the —SH group relates more to potential cell activity, rather than indicating active cell division as postulated.

6864

Physico-chemical Aspects of Sex in Plants.*

W. F. LOEHWING.

From the State University of Iowa.

The excellent review by Joyet-Lavergne[1] of the earlier work on this problem obviates the need of recapitulation here. It may be stated, however, that the formative responses of the sexes in dioecious plants under various conditions have been much more thoroughly recorded than their concomitant physiology. This investigation was undertaken in an attempt to secure comprehensive data concerning normal staminate and pistillate metabolism in

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[7] Slifer, E. H., *Physiol. Zool.*, 1930, **3**, 503; *J. Morph. and Physiol.*, 1931, **51**, 613.

[8] Bodine, J. H., *Physiol. Zool.*, 1932, **5**, 538.

* Aided by a grant from the National Research Council.

[1] Joyet-Lavergne, Ph., *La Physico-Chimie De La Sexualite.* Borntraeger, Berlin, 1931.

typical dioecious plants. For comparison of long and short day photoperiodic responses, data are presented for spinach and hemp as representative of these two types.

Plants were grown in the greenhouse in the early spring and summer in triplicate series which were thinned to between 50 and 100 plants each, depending upon the age at which they were to be analyzed. The vegetative-reproductive cycle was controlled by supplementing daylight with electric light as needed at the rate of two 100-watt lamps per square yard at the height of one foot above plants. It was necessary to lengthen the early spring daylight 6 to 7 hours per day to prevent young hemp from flowering prematurely. Otherwise all plants developed normally. Six to 10 plants of each series were analyzed in early flowering stages prior to shedding of pollen, and a similar number during incipient fruiting. Entire plants were gathered and divided into duplicate samples of whole tops and roots for analysis, the average of analyses of all series being reported. Each table thus represents data from a study of over 100 plants. Roots were washed free of soil, rinsed in distilled water and then dried for 30 minutes at room temperature. Spinach had begun to shift from the flat rosette of its vegetative phase to the shooting phase of the central axis preparatory to flowering when the first analyses were made. Male hemp plants had begun to show the formation of a leafless tip and the females the terminal rosette prior to flowering at the time the first materials were collected for analysis.

Conductivity and pH were determined electrometrically at 25°C. Indophenol-oxidase activity was measured colorimetrically at 25°C. as the amount of indophenol formed by 1 cc. of expressed sap per hour in 25 cc. portion of a freshly prepared solution made up as follows 0.144 gm. a-naphthol, 0.209 gm. dimethyl-paraphenylenediamine HCl, 0.1175 gm. anhydrous sodium carbonate, 10 cc. of 95% ethyl alcohol and 140 cc. of water. The sap test was compared with a 50% alcoholic color standard containing 0.353 gm. indophenol per liter and then corrected by deducting the reading of a 25 cc. blank without sap.

The analytical data for spinach (Table I) flowering under long day conditions, show the more rapid development of staminate plants in the earlier stages even though these are lower than females in dry weight. Total ash is also higher both in tops and roots throughout. Once sexual dimorphism has developed, there is practically no overlap of the sexes in ash or moisture content in

TABLE I.
Analyses of Prickly Seeded Spinach

	Age 8 weeks[a] Tops		Age 12 weeks[b] Tops		Age 12 weeks[b] Roots		Age 15 weeks[c] Tops	
	♂	♀	♂	♀	♂	♀	♂	♀
Fresh weight, gm.	2.30	2.60	20.60	15.00	7.40	6.90	33.41	31.08
Dry Weight, %	7.62	7.93	7.80	9.40	6.20	7.20	8.23	10.24
% of dry weight								
Total Ash	22.10	17.68	24.00	18.80	8.65	9.05	24.80	19.68
Iron	0.09	0.05	0.09	0.06	0.05	0.08	0.07	0.06
Calcium	2.92	2.87	3.00	2.00	0.60	1.33	2.20	1.63
Magnesium	0.64	0.12	0.88	0.40	0.22	0.80	0.99	0.78
Potassium	2.34	3.92	4.00	3.10	1.20	0.98	4.40	2.58
Phosphorus	0.78	0.21	0.60	0.39	0.40	0.27	0.58	0.42
Nitrogen	4.12	4.94	4.70	4.85	3.07	3.27	4.53	4.91
Sugars	3.59	3.20	3.84	3.30	3.00	2.20	2.62	3.34
Starch	11.10	8.45	10.70	9.90	21.10	14.42	11.52	10.32
Expressed sap								
pH	6.03	6.14	6.10	6.00	5.60	5.84	6.16	5.83
Osmotic Pressure[d]	11.58	11.58	15.12	12.50	6.95	5.80	14.87	13.63
Conductivity[e]	.0257	.0236	.0303	.0270	.0288	.0200	.0334	.0342
Gm. per liter of expressed sap								
Dry Weight	38.04	38.60	42.10	40.80	27.07	20.48	48.20	42.00
Total Ash	11.50	10.26	16.20	11.40	8.04	6.42	14.20	12.00
Sugars	5.37	4.10	6.46	3.55	4.80	3.65	2.36	3.05
Nitrogen	1.18	0.64	1.44	0.60	0.78	0.51	1.07	0.51
Indophenol[f]	36.39	42.78	66.20	42.90	44.87	26.09	61.33	43.46

[a] Formation of flower primordia commenced at time of analysis.
[b] Plants in full flower but little pollen shed.
[c] Most pollen shed and some young fruits started.
[d] Osmotic pressure in atmospheres.
[e] Specific conductivity in reciprocal ohms.
[f] Milligrams of indophenol formed per liter of sap per hour.

plants of comparable age. Practically all of the mineral nutrients are higher in staminate plants, although the differences between the sexes diminish as the males continue to shed pollen. This is especially true of iron and magnesium, of which the lowest values recorded in males were well above the highest found in females of similar age. Total nitrogen not including nitrates remains consistently slightly higher in females but with a tendency of extremely low females and high males to overlap as anthers mature. Due to the small variations in reducing and non-reducing sugars, only total sugars are reported. These along with starch are also significantly higher in males, except that pollen formation eventually reduces the sugar content of males below that of females in the early fruiting stages.

On the whole, the rate of mineral absorption by males exceeds that of females and translocation from roots to tops also occurs more rapidly. In young plants insoluble calcium, magnesium and iron, for example, tend to accumulate in roots of females, evidenced by the fact that even though the total ash content of female roots is higher than in males, soluble salts in expressed sap are lower. Probably the most significant difference between the sexes in spinach is the larger amount of soluble material in the male sap, shown by its higher osmotic pressure as well as by its greater ash, sugar and nitrogen content. Staminate tissue fluids also maintain a higher specific conductivity and greater oxidase activity than females. Despite variations of individual plants, even the lowest rates of indophenol formation by oxidase were above those of the most active females.

These facts all reflect the more rapid metabolism of the staminate plants, necessitated in part by the need for rapid elaboration of relatively large amounts of pollen often comprising 10 to 15% of the entire dry weight of the tops. The celerity of staminate development correlates well with the rapidity of nutrient absorption, especially of iron which is important in oxidase activity and of phosphorus which presages early maturation of males.

In view of the importance of spinach as a source of iron in the diet and the fact that the human assimilation of nutrients is favored by their solubility in the vegetable foods ingested, the established commercial practice of thinning the spinach crop is unfortunate because it involves essentially the elimination of males which are the more desirable in both respects.

Hemp (Table II), normally flowering under short day condi-

TABLE II.
Analyses of Common Hemp, *Cannabis sativa.*

	Age 5 weeks[a]		Age 7 weeks				Age 10 weeks	
	Tops	Roots	Tops		Roots		Tops	
	Veg.	Veg.	♂	♀	♂	♀	♂	♀
Height of tops, cm.	65.00		90.00	80.00			90.00	91.20
Fresh Weight, gm.	9.77	0.81	36.85	48.68	2.38	4.45	80.00	67.62
Dry Weight, %	20.30	15.41	20.84	18.80	21.10	17.87	23.34	24.09
			% of dry weight					
Total Ash	14.08	11.06	17.25	17.70	8.08	9.52	15.00	18.38
Iron	0.015	0.079	0.029	0.009	0.030	0.042	0.039	0.031
Calcium	4.66	1.11	5.24	5.04	0.90	0.92	5.04	5.30
Magnesium	0.30	0.15	0.42	0.21	0.27	0.34	0.26	0.53
Potassium	1.75	2.24	1.31	1.45	1.07	1.17	0.66	1.08
Phosphorus	0.47	0.53	0.54	0.50	0.30	0.36	0.47	0.60
Nitrogen	3.98	2.18	3.49	3.65	1.99	1.64	3.61	3.69
Sugars	4.40	3.31	4.49	3.39	5.32	4.92	7.40	6.10
Starch	10.07	17.04	13.52	17.89	17.62	19.50	12.63	16.45
			Expressed sap					
pH	6.81	6.11	6.38	7.23	6.20	5.95	7.49	7.02
Osmotic pressure	15.32	10.49	14.59	12.90	12.18	10.25	16.30	15.80
Conductivity	.0253	.0218	.0268	.0295	.0231	.0293	.0209	.0216
			Gm. per liter of expressed sap					
Dry Weight	61.00	39.40	81.80	70.75	42.80	51.40	99.54	87.09
Total Ash	11.45	7.77	12.53	12.45	9.84	9.46	12.38	8.22
Sugars	19.50	4.80	10.38	7.93	9.40	7.39	20.87	17.96
Nitrogen	3.10	0.66	5.85	5.75	2.90	2.84	7.05	5.24
Indophenol	73.67	29.45	88.25	73.67	40.40	27.57	52.00	51.86

[a] Vigorously vegetative plants showing no sex dimorphism. See corresponding footnotes to Table I.

tions of late summer, shows fewer contrasts between the sexes than observed in spinach, due probably to the fact that the life span of the 2 sexes is more nearly equal in hemp. In view of the differences in length of vegetative activity in spinach and hemp, the common characteristics of their corresponding sexes probably constitute the chief physico-chemical differences of staminate and pistillate metabolism.

Staminate hemp is consistently higher in percentage of dry weight until considerable quantities of pollen have been shed. Though differences in percentage of total ash in tops are inappreciable during flowering stages the iron and magnesium content of males is nevertheless greater, with practically no evidence of overlap between sexes. Total nitrogen and sugars of tops and roots are also higher in males as are total solids dissolved in sap including ash, sugars and nitrogen. These conditions are clearly reflected in the osmotic pressure and specific conductivity of body fluids when analyses are averaged but not always this definitely in individual cases. Phenoloxidase activity remains higher in staminate plants until they near the close of their activity after flowering. These metabolic conditions all correlate excellently with the known precocity of staminate hemp plants.

In summary, it may be noted that staminate plants of photoperiodic, long day spinach and short day hemp correspond in having higher percentage of iron, magnesium and sugar, more soluble sap constituents, and greater oxidase activity of tissue fluids.

Western New York Section.

Cornell University, May 20, 1933.

6865

Action of Thyroxin on Tissue Respiration.*

J. A. DYE.

From the Department of Physiology, Cornell University.

Experiments in which thyroxin, or thyroid substance, has been added directly to surviving animal tissues *in vitro* have given, almost without exception, negative results. This might have been expected since the maximum action from a single dose of thyroxin *in vivo* is reached only after several days while the tissue survival is for about an equal number of hours. Respiration experiments on tissues taken from cretin and thyroxinized animals have given variable results. The reports of several workers,[1-7] however, offer rather convincing proof of a direct thermogenic action of thyroxin on the body tissues. The present work was begun to study the nature of this thermogenic action.

The oxygen consumption (QO_2) of muscle fasciculi was measured volumetrically by means of differential microrespirometers (Fenn). The animals were routinely anesthetized with amytal for 30 minutes after which they were bled from the carotid artery. The left semimembranous muscle was quickly removed and the muscle strips carefully prepared according to the method of Richardson,

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* This work was aided by a grant from the Heckscher Research Foundation of Cornell University.

[1] Rohrer, A., *Biochem. Z.*, 1924, **145**, 154.

[2] Hopping, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 726.

[3] Gerard, R. W., and McIntyre, M., *Am. J. Physiol.*, 1933, **103**, 225.

[4] Adler, L., and Lipschitz, W., *Arch. f. exp. Path. u. Pharm.*, 1922, **95**, 181, 206, 225, 236.

[5] Neuschloss, S. M., *Klin. Wochenschr.*, 1924, **3**, 57.

[6] Ahlgren, G., *Skan. arch. f. Phys.*, 1925, **47**, supp. 225.

[7] Dye, J. A., and Maughan, G. H., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 439, 441.

Shorr, and Loebel.[8] These were suspended in the microrespirometer flasks in buffered saline, pH 7.35, or in the same solution with 0.9 mg. of lactic acid per cc. The air in the flasks was replaced with oxygen. The CO_2 was absorbed by 5 drops of N NaOH solution. The time interval before the microrespirometers were placed in the water bath was 35 minutes. A temperature equilibration of 30 minutes was always allowed (bath T. 38°C.). The tests usually ran for 7 hours. Bacteriological precautions were carefully taken. The tissues were later dried to constant weight at 110°C.

A 36-hour fast in normal pups diminishes the QO_2 of surviving muscle strips by about 21% when compared with that of non-fasting animals. Those of fasting normal animals consumed 2.73 cmm., those of thyroidectomized animals 2.06 cmm. per mg. of dry tissue per hour. Whether fasting or not the QO_2 of muscles from thyroidectomized pups is reduced by about 24.2%. When glucose, but not lactic acid, is added, this reduction in oxygen consumption is only 20%. This might indicate that these tissues handle glucose better than lactic acid. An increase in QO_2 varying from 4 to 9% is obtained by the addition of lactic acid and glucose to the suspension fluid.

The QO_2 values for similar muscle strips taken from pups which had been fed thyroxin tablets (Squibbs, daily; a total of 168 mg. in 35 days) show a comparable oxygen consumption of 4.07 cmm. This represents a 50% increase for thyroxinized animals' muscle strips (first hour) when suspended in saline. When lactic acid was added this increase was only 36.6%. The QO_2 percentages, however, differed in the 2 groups of animals during the succeeding hours. The decline in QO_2 was relatively steeper in the tissues from thyroxinized animals whether or not lactic acid was added. The same gradient decline was steeper in tissues suspended in saline whether the animal was thyroxinized or not, and was greater in thyroxinized than in control animals. The QO_2 of thyroxinized tissue during the seventh hour was only 21.1% greater for saline and only 5.9% greater for lactic acid than in the normal.

These results seem to be explained best by assuming two facts: (1) Thyroxin feeding increases the oxidative capacity of muscle, probably by increasing the tissue oxidases, and (2) due to this increased oxidative capacity the available substrate is exhausted more rapidly. This is shown exceptionally well by the tissues from one thyroxinized animal which gave a QO_2 of 4.28 cm. per mg. of dry tissue per hour for the first hour, but which fell off 61.7%, 25.5%, and 4.8% during the second, third, and fourth hours, respectively. In the present series of experiments the average rates of decline per hour were 7.5% for normal and 8.8% for thyroxinized animals. When lactic acid was added these declines were somewhat lower, 6% and 7.9% respectively.

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[8] Richardson, H. B., Shorr, E., and Loebel, R. O., *J. Biol. Chem.*, 1930, **86**, 551.

The possibility of thyroxin acting as a catalyst is not ruled out, but evidence in agreement with the above expressed point of view is found in the results of Ahlgren,[6] Adler and Lipschitz,[4] Neuschloss,[5] and by previous work from this laboratory.[7, 9]

6866

Adrenal Cortical Hormone and Tissue Respiration.*

J. A. DYE.

From the Department of Physiology, Cornell University.

Aub, Forman, and Bright[1] reported a 25% decrease in basal metabolism in totally adrenalectomized cats. Injections of extracts at that time available were without effect in increasing this diminished metabolism.[2] Variable results have been reported by others.[3–6] Swingle, Pfiffner, and Webster[7] have shown that the basal metabolism of bilaterally adrenalectomized cats falls progressively from the 6th day postoperative to a level approximately 50% below normal when the animal becomes prostrated. Administrations of adrenal cortical extract to prostrate animals raises the metabolism within 24 to 48 hours, this may reach a point from 10 to 18% above normal in 48 to 72 hours. Upon discontinuing the injections the metabolism returned to normal. These same

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[9] Dye, J. A., and Waggener, R. A., *Am. J. Physiol.*, 1928, **85**, 1, 365.

* This work was aided by a grant from the Heckscher Research Foundation of Cornell University.

[1] Aub, J. C., Forman, J., and Bright, E. M., *Am. J. Physiol.*, 1922, **61**, 326.

[2] Aub, J. C., Bright, E. M., and Forman, J., *Am. J. Physiol.*, 1922, **61**, 249.

[3] Golyakowski, Vrach., St. Petersburg, 22, 1017.

[4] Marine, D., and Bauman, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

[5] Scott, W. J. M., *J. Exp. Med.*, 1922, **36**, 199.

[6] Gracinescu, A. V., *Pflüger's Arch.*, 1913, **152**, 187.

[7] Swingle, W. W., Pfiffner, J. J., and Webster, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 728.

changes occurred in animals previously thyroidectomized.[8] Subcutaneous injections of large amounts of cortical hormone were without effect in increasing the respiratory metabolism of normal cats or rabbits, but similar injections in thyroidectomized animals increased metabolism 15 to 30% in 80% of the animals. They suggest that the cortical hormone possibly acts directly on the oxidation-reduction processes which occur within the tissues themselves, an action which is more rapid than that of thyroxin.

Engle, Britton, and Kine[9] were able to demonstrate that injections of adrenal cortical hormone increased the endurance capacity and energy output of treadmill-exercised dogs by about 100%. This effect persisted for 10 to 15 days.

The present investigation was begun to determine whether the cortical hormone has any effect on the QO_2 of surviving muscle fasciculi. Fasting cats were given single intraperitoneal injections of 5 to 10 cc. of eschatin after which they were permitted to survive for periods varying from 2.5 to 6 hours. They were killed by bleeding through the carotid artery under amytal anesthesia. One animal was given an injection of 2.5 cc. of eschatin with a second similar dose on the third day and was sacrificed 7 days later. The general methods were identical with those described in the preceding paper.

These results show no consistent differences in the QO_2 of the tissues from treated and non-treated animals under the present dosages and survivals. The averages for 7 eschatin treated and 2 control animals were 1.97 cm. and 1.98 cm. per mg. of dry tissue per hour, respectively. From 2 experiments on totally adrenalectomized cats, one of which died on the 9th day and the other was sacrificed while in coma on the 11th day postoperative, the QO_2 values were found to be no lower than could be accounted for by the long premortal fast of the animals. When suspended in saline, where the tissue must depend upon its own substrates, the values were 1.92 cm. and 1.70 cm. for normal and adrenalectomized animals, respectively. When, however, an artificial substrate was added in the form of sodium lactate (0.9 mg. per cc.), the values were 1.90 cm. and 2.08 cm. respectively. There would seem to be no inability of the tissue to oxidize a substrate if one were present. This evidence supports the conclusion that the adrenal cortical hormone has no direct action on the oxidation-reduction processes of the tissues themselves.

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[8] Webster, B., Pfiffner, J. J., and Swingle, W. W., *Am. J. Physiol.*, 1932, **99**, 710.

[9] Engle, E., Britton, S. W., and Kline, R., *Am. J. Physiol.*, 1932, **102**, 707.

6867

Effect of Various Bacteria Upon Growth of Planarian Worms.

ALICE M. BAHRS. (Introduced by H. J. Sears.)

From the Department of Bacteriology, University of Oregon Medical School.

Dr. Wulzen and the author have been engaged over a period of years in testing the growth-promoting power, for planarian worms, of the tissues from various animals. We have found that the growth response of these worms varies markedly when different tissues[1] or the same tissue from animals reared under different conditions,[2] are fed as the entire diet.

As bacteria produce definite effects upon the growth of mammals when the tissues are invaded, it was of interest to test the effect upon planarian worms of adding various bacteria to a food of known growth-promoting power. The following organisms were selected: 2 strains of *Staphylococcus aureus* (No. 47P, isolated from raw beef liver, and No. 6, from a human case of mastoiditis), *Proteus vulgaris, Bacillus subtilis,* and *Sarcina lutea,* all grown upon plain agar slants for from 48 to 72 hr. before being fed to the worms.

The technique used in planarian nutritional studies has been described.[1, 2] Each experimental diet was fed to a group of 30 measured worms, over a period of one month. The worms were kept in finger bowls, in sterilized tap water, at a temperature of 25°C. The experimental groups of worms were fed twice per week, the food being allowed to remain in the dishes with the worms for about 3 hours. After feeding, the excess food was removed and the worms washed in several changes of sterilized tap water.

In these experiments raw beef liver, an excellent food for planarian worms, was used as the basal diet. It was made into a fine brei by mashing upon a glass plate and removing connective tissue and blood vessels. One gram portions of liver brei were weighed out and to each of these portions was added the particular strain of bacteria to be tested. Two agar slants of organisms were added to each portion of liver, the organisms being scraped from the slants with a sterile loop. The bacteria were thoroughly blended with the liver brei upon a glass plate by means of a flexible spatula, and after being thus mixed the mass was dropped into the dish with the experimental group of worms. There is no doubt that the worms did ingest the bacteria along with the liver, for the intense yellow color of *Sarcina lutea* could be seen in the worms after they had eaten a mixture of liver and this organism. The other organisms were not sufficiently colored to make this apparent, but all mixtures of liver and bacteria were readily eaten by the worms and their gorged appearance after feeding left no doubt as to their success with the food.

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[1] Wulzen, Rosalind, *Univ. of Calif. Pub. Physiol.*, 1926, **7**, 1.

[2] Bahrs, Alice M., *Physiol. Zoology*, 1931, **4**, 189.

At the end of 4 weeks the total gains in length for the experimental groups of worms fed the following diets were: liver brei + *Sarcina lutea,* 169 mm.; liver brei + *Bacillus subtilis,* 164 mm.; liver brei, 163 mm.; liver brei + *Proteus vulgaris,* 148 mm.; liver brei + *Staphylococcus aureus* No. 47P, 139 mm.; liver brei + *Staphylococcus aureus* No. 6, 129 mm.

We have considered a difference of 30 mm. between 2 groups of worms significant. Treating the figures statistically, this appears to be a true difference. There is a difference of 15 mm. between the total lengths of the group fed liver brei and liver brei + *Proteus vulgaris,* and this difference is 3.3 times the probable error, not a significant difference. A difference of 24 mm. exists between the total gains in length of the worms fed liver brei and liver brei + *Staphylococcus aureus* No. 47P, 3.7 times the probable error, not a significant difference. 34 mm. is the difference between the total gains in length of the worms fed liver brei and liver brei + *Staphylococcus aureus* No. 6, 5.6 times the probable error, a statistically significant difference. (If the difference is 4 times the probable error of the mean, it is considered statistically significant.)

Two tests were made upon *Staphylococcus aureus* No. 47P. In the first experiment the liver control and experimental group of worms had a total length of 249 and 246 mm. respectively. Four slants of *Staphylococcus aureus* No. 47P were added to the gram portion of liver fed to the experimental group of worms. At the end of 4 weeks these worms had a total gain in length of 169 mm., or 62 mm. less than the experimental group fed liver alone. This experiment shows that a larger number of bacteria had a more marked effect upon the growth of the worms, and also that the experiments can be duplicated.

None of *these* organisms produced pathological changes in the worms. Natural infections among planarian worms do occur, if care is not exercised to avoid outside contamination. We began using only sterilized water upon the worms about five years ago, and since then have had no trouble from these epidemics. Very definite pathological lesions are produced in the worms during these epidemics, but whether these are of bacterial or protozoan origin I do not know, and I doubt whether any work has been done upon this subject.

6868

Pellagra: An Experimental Study of the Skin Lesions.

TOM DOUGLAS SPIES. (Introduced by Joseph T. Wearn.)

From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Western Reserve University, and the Medical Service, Lakeside Hospital, Cleveland, Ohio.

It was reported in a previous publication[1] that the skin lesions of pellagrins improved while they were restricted to a so-called "pellagra-producing" diet. Since this diet consisted of such diverse foods as cornmeal, pork fat, artificially colored (synthetic) maple syrup, polished rice, cornstarch pudding, and sugar, it seemed advisable to observe the skin lesions of pellagrins limited to a diet less complex in food materials and even more deficient in vitamin G. Evidence is presented in this paper to show that the dermatitis of pellagra often improves while the patient is restricted to cornstarch and lactose.

Ten cases with the characteristic dermatitis of pellagra were chosen for this experiment and extreme care was exercised to select patients without neurological involvement. Each patient remained in bed during the experiment and was given a diet of cornstarch and lactose amounting to 2,000 or 3,000 calories per day. Two of the 6 individuals in this series with stomatitis were unable to tolerate this diet so it was discontinued. The skin lesions of the 8 patients who remained on the diet of lactose and cornstarch showed definite improvement during the first 6 to 15 days of the experiment. The stomatitis of one individual improved and that of the 3 others became worse while they received the restricted diet. The 4 patients free of stomatitis at the beginning of the experiment did not develop it and none of the pellagrins used in this experiment showed any signs of central nervous system involvement.

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[1] Spies, T. D., *Am. J. Med. Sc.*, 1932, **184**, 837.

It has been noted by the writer[2] as well as by others that pellagra may involve the central nervous system, the gastro-intestinal tract, and the skin in the same patient, while in other patients it may affect one or 2 organ systems. In several instances[2] the writer has observed that the dermatitis and stomatitis healed while there was a steady progression of the central nervous system disease. It seems from these observations that too much importance may have been attached to the healing of the skin lesions by some of the investigators of the past. In view of this work and of the known observations concerning the lack of uniformity of the disease in involving the various organ systems of the body, it even appears possible that the specific chemical substance related to the development of the dermatitis is not the same as that affecting the other manifestations of pellagra.

6869

Protein as a Stimulant for Secretion of Pepsin.

GEORGE R. COWGILL AND ELIZABETH R. B. SMITH.*

From the Department of Physiological Chemistry, Yale University, New Haven, Connecticut.

Experiments were conducted on the secretion of gastric juice by Pavlov gastric pouch dogs in response to diets in which the protein was made the variable of interest. These diets were adequate for maintenance and differed only with respect to the kind and amount of protein present. Secretion was followed for 9 hours subsequent to feeding. Gastric juice samples were examined for free and combined acid by titration, chlorides by the method of Van Slyke,[1] and peptic activity by the procedure of Gilman and Cowgill.[2]

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[2] Spies, T. D., unpublished data.

* This is a report of a part of the data contained in the dissertation presented by Elizabeth R. B. Smith to the Faculty of the Graduate School, Yale University, May, 1933, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The expenses of this research were defrayed in part by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry of the American Medical Association.

The following proteins were tested: commercial casein (2 levels of concentration in the diet), meat residue** (2 levels), lactalbumin,† hemoglobin,‡ mixed serum proteins, coagulated egg white, edestin,¶ and mixed proteins as combined in a commercial dog food.§

Total volumes of gastric juice secreted and rate of secretion varied widely, but, with a given protein, the total amount secreted was roughly proportional to the percentage of protein in the diet. Titratable acidities followed closely the rate of secretion as has been described by Pavlov[3] and many others. Total chloride concentrations were constant within the range of about 150 to 160 milli-equivalents per liter, thus confirming the observations of Gamble and McIver,[4] of MacLean and others,[5] and of Gilman.[6]

The present results are not directly comparable with those of Pavlov[3] because of differences in methods of estimation and calculation. It is interesting, however, to note that the observations made in this study differ from those of Pavlov in that the total pepsin secreted per hour (the product of the volume of juice in cubic centimeters and the peptic activity in units) maintained a remarkably constant value for each dog after the first hour of the post-prandial period. These results would tend to indicate that (a) the rate of secretion and, therefore, the amount of acid are markedly affected by the character and amount of protein fed, but that (b) the secretion of pepsin is almost as strictly a constant as the concentration of total chloride. Total pepsins for the first hour tended to be higher than those of the later periods. This can readily be explained as the result of a flushing effect on the glands, similar to that observed with histamine injection as reported by Vineberg and

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[1] Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1923, **58**, 523.

[2] Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 743.

** Obtained from the Valentine Meat Juice Co., Richmond, Va.

† Obtained from the Casein Co. of America, New York City.

‡ Obtained from the Eastman Kodak Co., Rochester, N. Y.

¶ Obtained from the Pfanstiehl Chemical Co., Waukegan, Ill.

§ Bal-Ra, prepared by the Richmond Abattoir, Richmond, Va.

[3] Pavlov, I. P., The Work of the Digestive Glands, London, 1910.

[4] Gamble, J. L., and McIver, M. A., *J. Exp. Med.*, 1928, **48**, 837.

[5] MacLean, H., Griffiths, W. J., and Williams, B. W., *J. Physiol.*, 1928, **65**, 77.

[6] Gilman, A., Dissertation, Yale University, 1931.

Babkin[7] and by Gilman and Cowgill.[8] Ten experimental dogs were used in the course of the 11 diets studied; 3 of the animals were used on every ration studied; the tests were confirmed by further studies on from one to 5 other dogs.

6870

Vitamin A Deficiency in Cattle Under Natural Conditions.*

G. H. HART, S. W. MEAD AND H. R. GUILBERT.

From the College of Agriculture, University of California, Davis.

On California ranges during the dry feed season, conditions are favorable for the development of vitamin A deficiency in livestock. Under average conditions, however, the length of the dry season coupled with the varied sources of feed containing some vitamin A is such that the reserves accumulated in the bodies of the animals from the green feed season are sufficient to carry them through the drought period. While its existence has been suspected during recent years, the past winter has been the first time the diagnosis has been definitely established. This was possible because weather conditions and management of one ranch in southern Tulare County caused its development in such a severe form that 100 animals died with many more showing symptoms in a herd of about 250 head.

The ranch comprises 3000 acres of land practically all of which is farmed to wheat and barley. The owner maintains 75 dairy cows. Calves from these cows, together with others purchased when a few days old from dairy farmers, nurse these animals. The calves are finally sold for veal.

In the spring of 1932 rains ceased early, resulting in the feed being dry and grain fields yellow by May 15. In the fall the first rains came about the middle of December and were followed by such cold weather that green feed did not become available until the middle of February, 1933.

During this unusually long dry feed period, of 9 months, part of the cattle grazed the unharvested grain fields through the summer. Thirty-five of the cows were left on a leased adjoining natural range until August 10th and then placed on stubble from which wheat and barley had been harvested. Early in the fall and through the winter they were fed roughage from a stack adjoining the corral. This consisted of straw mixed with wheat and barley hay showing some traces of green color. The hay came from the borders of the fields that were harvested for grain. Barley was the main concentrate fed. This was later supplemented with whole cottonseed. A group of about 50 steers purchased in October was brought in from native grass range and fed in dry lot. These steers, in addition to the straw, grain hay and barley, received 2 pounds daily of a proprietary concentrate feed containing 12% protein.

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[7] Vineberg, A. M., and Babkin, B. P., *Am. J. Physiol.*, 1931, **97**, 69.

[8] Gilman, A., and Cowgill, G. R., *Am. J. Physiol.*, 1931, **97**, 124.

* This report is part of an investigation on the relation of nutrition to reproduction in livestock which became cooperative with the United States Bureau of Animal Industry, July 1, 1929.

The deficiency manifestations were less marked in the adult cows than in the young stock. The general condition of the cows was poor, although 6 to 7 pounds of barley were being fed per head daily in addition to the straw and grain hay. When 2 parts of cottonseed and one part of barley were substituted for the barley alone there was evidence of improvement, but deficiency symptoms were not relieved. The most interesting symptom was night blindness, noticed in the twilight and after darkness when an electric light was turned on in the corral. If attempts were made to handle the cattle at this time they ran into each other, or objects in front of them, and became nervous and excitable. A few showed ophthalmia with ulceration of the cornea. The milk and cream from the animals entirely lacked color as did butter made from the latter.

These cows started to calve the latter part of August. The early calves were normal and although they became unthrifty, did not show definite symptoms until about 12 weeks of age. Birth of normal calves continued until late in the fall. During the months of December and January, however, when 25 to 30 cows calved, all of the calves were born weak, developed severe diarrhea and died when one to 5 days old. At this same time, calves purchased from dairymen on alfalfa ranches remained normal for 6 to 8 weeks after being brought on the ranch and nursing the same cows. This indicated the non-infectious nature of the diarrhea. One bull was kept on the ranch and although he was in poor condition during the winter, we do not have data regarding his fertility. Ability to serve cows was not completely lost. Many of the cows did not come in estrum for 5 or 6 months after calving, when green feed became available.

The feed lot steers varied in age from yearlings to 2-year-olds. The younger cattle were mostly affected. Eye lesions were the principal symptoms beginning with profuse lacrymation followed by clouding and ulceration of the cornea.

The young animals, varying in age from a few weeks to one year, showed the most marked and varied symptoms and the mortality was high. The eye lesions varied from profuse lacrymation and slight clouding of the cornea to extensive ulceration of the cornea, loss of aqueous humor, lens opacity and shrinking of the eyeball with complete and permanent blindness.

The appetites of the animals were capricious, there was intermittent diarrhea, general unthrifty appearance and pulmonary complications. With periods of inclement weather and exposure, pneumonia was the terminal condition causing death in nearly all cases.

Liver samples from 4 of the dead animals were brought to the laboratory, the liver oil extracted and tested with antimony trichloride after the method of Carr and Price[1] for the colorimetric determination of vitamin A. In all cases solutions representing as much as 10 gm. samples of liver tissue were negative. As we have demonstrated positive tests with solutions representing as little as 1 mg. of adult bovine liver tissue, we feel that the negative reaction with this large test quantity was evidence that the liver tissue in these cases contained no vitamin A.

Three animals moved to University Farm. On February 28, three animals were taken to Davis. No. 1 was a steer born on the ranch in March, 1932, and was weaned September 1. After weaning it was fed straw, grain hay, barley and salt. It first showed symptoms early in January. On February 28 it was extremely gaunt, totally blind, discharging profusely from the nostrils, quite unsteady on its legs and lacked appetite. Drenching with reconstituted dried skim milk was resorted to and in addition 30 mils of good quality cod liver oil was given daily. After 4 days the appetite began to improve, the discharge from the nostrils ceased and the muzzle became moist. It was continued on straw, barley and cottonseed meal plus the cod liver oil and made a complete recovery except for the loss of sight.

No. 2 was a steer born December 1, 1932. It came from an alfalfa ranch and grew normally during the first 6 weeks. While still nursing it developed severe diarrhea and eye lesions and showed evidence of lung involvement on January 19. On February 28, it was extremely emaciated, the left cornea was badly ulcerated, the right cornea clouded and respiration was rapid. It was selected for pathological study without any thought of its surviving. It died on March 3, and showed extreme lung involvement which had gradually progressed over a period of 6 or 7 weeks to be the final cause of death.

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[1] Carr, F. H., and Price, E. A., *Biochem. J.*, 1926, **20**, 497.

No. 3 was a Holstein heifer born on the ranch November 15, 1932, and was still nursing its dam. The animal was in nearly normal condition of flesh but the right eye was badly affected with severe ulceration of the cornea. On February 28, its appetite was good and it was kept on the same diet as it had received at the ranch except that reconstituted dried skim milk was substituted for the milk of its dam. No cod liver oil was given. It gradually became worse, showing emaciation, intermittent diarrhea, irregular appetite and some evidence of lung involvement by rapid breathing under slight exertion. The regression of the animal was very gradual, considering the low vitamin A intake and its condition on arrival. It was killed March 10, in order to have fresh tissues for pathological study.

All of the animals on the ranch have recovered since the advent of green feed except for blindness in 2 cases, which will be permanent. This heavy loss could probably have been entirely obviated had the owner purchased alfalfa hay instead of the cottonseed in the fall.

The feeds on this ranch were inadequate in vitamin A, rather than completely devoid of it. The diet of the cattle was also low in calcium. The liberal feeding of grain, coupled with adequate protein when cottonseed was added, stimulated growth and milk production and is probably responsible for the severe incidence of deficiency manifestations. Under natural range conditions with deficient protein, phosphorus and low energy intake, the manifestations have been limited largely to reproductive difficulties, although the dry range forage is probably equally low in vitamin A.

6871

A Feeding Tube for Quantitative Dietary Experiments on Rats.

M. L. TAINTER.

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco.

A difficulty in conducting feeding experiments on rats, when the amount of food consumed is a factor, is that the rats spill or waste varying amounts of the food if it is kept in the usual types of containers. We have devised a simple, inexpensive feeding tube from which rats do not waste or lose food, and with which quantitative experiments can be readily carried out. Being made entirely of glass, it has the further advantages of transparency, and of being suited for experiments where various drugs, which might react with metals, are present in the diet.

The feeding tube is made from glass tubing, 2 inches in diame-

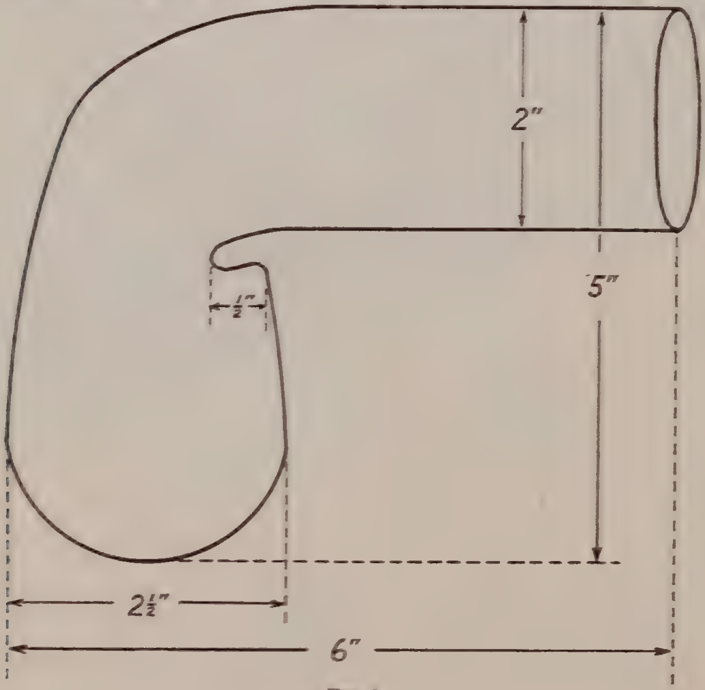

FIG. 1.

ter, which is bent into a right angle and the lower end sealed off. The accompanying figure indicates the shape and dimensions of the tube. An important feature is the small shelf projecting into the lumen at the bend. This is made by accentuating the wrinkle produced when the tube is bent, and shaping it in the direction and form indicated. This serves to catch any food the rats might scratch out, and to cause it to drop back into the bulb. The bulb as drawn will hold about 75 gm. of diet. Its size may be altered by blowing it larger or smaller as might be needed for any special purpose. Tubing of the diameter illustrated here may be used with rats weighing up to 250 gm. If larger animals are to be used, a tubing of larger diameter would be desirable.

6872

Experiments on the Use of the K Medium.

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One of the most striking of recent contributions on bacterial variability has been that of Kendall,[1] who reported the preparation of a special medium in which ordinary bacteria developed a filterable or "virus-like" stage. A number of the commoner bacteria, when inoculated into this medium, were converted within a short time to a form which passed Berkefeld filters and which could not be cultivated on ordinary media. Not only was the "K" medium effective in promoting such a change, but it was claimed to be effective also in bringing about the opposite process, namely reversion from the filterable form to the usual visible organism.

Since such a method, if effective, would place in our hands a valuable tool for the study of many debated problems on bacterial variability, an investigation of the K medium was undertaken in this laboratory.

Several different lots of this medium were employed. One was prepared from hog intestine according to Kendall's directions. After the alcohol and benzene extractions, the preparation was dried, ground and kept in a stoppered container. At the time of use, the proper quantity of the powder was placed in test tubes or flasks and Tyrode solution added to give a 2% suspension. The pH of the medium was adjusted to 7.4. After autoclave sterilization the medium was somewhat turbid with considerable sediment. In addition to our own preparation, 2 experimental lots prepared by the Difco laboratories were used. The first of these, also prepared strictly according to Kendall's directions, produced a medium quite similar in appearance to our own. The second had been subjected to a more thorough extraction and washing and resulted in a much clearer medium.

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[1] Kendall, *Northwestern University Bull.*, 1931, **32**, No. 5, 8.

With these different preparations an attempt was made to convert several of the common bacteria to a filterable stage and then, after filtration, to cultivate them in the K medium and again establish growth on ordinary media. Laboratory stock cultures of the typhoid bacillus and of *Staphylococcus aureus* were used.

Three different procedures were employed. In the first series of experiments cultures were inoculated into 150 cc. amounts of K medium in flasks and held at 37°C. Samples were withdrawn for filtration after 48 hours, one week, and after 3 to 4 weeks. In the second series of experiments each culture was subjected to 5 successive transplants at 3-day intervals in the K medium. Following the incubation period each culture was held in the ice box until the series was completed, at which time the last 3 K medium tubes of each series were combined for filtration.

In the last series, repeated transfers in the K medium were also employed but in this instance transfers were made daily for 12 days. After transfer each culture tube was held in the ice box. Three separate filtration tests were made on each of these series of K medium cultures: the first 3 tubes were combined and filtered, then the middle 3 of the series (numbers 5, 6 and 7) and finally the last 3. This gave filtrates of cultures which had been passed through varying numbers of transfers in K medium.

Both *Eberthella typhi* and the *Staphylococcus* developed readily in the several different lots of K medium and produced a distinct clouding within 24 hours. In a number of cases agar plates were made from the K medium for purposes of colony study. The colonies which developed were uniformly smooth colonies. Rough colonies or the G forms of Hadley did not appear.

Microscopic examination of the cultures in K medium likewise failed to reveal any real departure from the usual morphological appearance and staining reactions of these organisms. In one series of experiments dark-field examinations were made of the typhoid bacillus and the staphylococcus after 12 successive transfers in the K medium. The organisms, however, presented much the same appearance as controls in the nutrient broth. Dark field examinations impressed us as being rather unsatisfactory since many particles of various sizes are to be seen in the turbid K medium, whether sterile or after development of a culture.

For filtration tests, both Berkefeld N and Seitz filters were used. Since the Berkefeld has been the standard type of filter chief significance was attached to it and the Seitz filters were used more as a matter of interest. A preliminary test of all filters was made by applying 24-hour broth cultures of *Serratia marcescens* and filters were not used unless the filtrate proved to be sterile. Altogether, 48 filtrations of K medium cultures were made with Berkefeld N candles and 24 with Seitz filters.

In testing for the presence of filter-passing forms, the K medium filtrates were usually examined as follows: 5 cc., 2 cc. and 0.25 cc. amounts of filtrate were inoculated into separate K medium tubes and also into tubes of dextrose veal infusion broth. A few drops were also placed on the center of an agar plate and the material transferred from plate to plate every 48 hours after the manner of the Hauduroy technic. Each plate was examined with the microscope before transfer was made to the next plate.

Growth could not be detected in any of these media, except on a few occasions when the filters were evidently at fault and the normal form of the typhoid bacillus appeared in all cultures within 24 hours. The tiny dew drop colonies which are supposed to represent an early stage in reversion from the filter-passing form to the normal were not seen in any of our experiments.

Our results, therefore, failed to confirm those of Kendall and we could obtain no evidence that the K medium was effective in promoting the appearance of a filterable stage of the organisms which we employed. This conclusion agrees with several other reports which have recently appeared.[2]

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[2] Craig and Johns, Proc. Soc. Exp. Biol. and Med., 1932, **29**, 661. Varney and Bronfenbrenner, *Ibid.*, 1932, **29**, 804. Seastone and Lawrence, *J. Infect. Dis.*, 1933, **52**, 20. Carpenter and Long, *J. Bact.*, 1933, **25**, 241.

6873

Fall of Infectivity and Immunizing Power of Poliomyelitic Tissue at Intervals after Complete Paralysis.*

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Monkeys infected with the virus of poliomyelitis, develop after the height of paralysis, sub-normal temperature, difficulty in deglutition, inanition and bed sores and so it is extremely difficult to maintain them alive for long periods of time. However, by wrapping them in blankets, bottle feeding and other measures, it was possible to keep a small number alive for varying intervals after complete paralysis. The cord of these animals was tested for infectivity by titrating a 5% or 10% suspension made up of 6 to 8 segments. During the course of this work, the infectivity of cord removed at the height of paralysis varied between 0.000625 and 0.0025 cc. of a 5% suspension. The results are shown in Table I.

TABLE I.

Cord Specimen: Interval after paralysis days	Infective dose in cc. of 5% suspension
2 (specimen 1)	0.01
2 (" 2)	0.005
4 (" 1)	0.1
4 (" 2)	0.1
6	0.15
8	0.2
11–12	0.5
16	0.8
19	3 cc. of a 15% suspension
21	failed to infect
23	" "

Further tests were made with 23-day-old cords, using the double inoculation technique.[1] One animal was inoculated intracerebrally with 1 cc. of a 20% suspension and 10 days later it again received 1 cc. intracerebrally and also 6 cc. intraperitoneally. This animal developed no symptoms of the disease. A second monkey was given 23-day cord which had been washed as free as possible of antibody. The animal from which it had been obtained was, after bleeding, infused with 2 liters of Ringer's solution. This, rather than isotonic gum acacia solution was used, for it was found that the latter sometimes reduced the infective power of spinal cord. One cc. intracerebrally and 6 cc. intraperitoneally of a 20% suspension and repeated in 11 days, gave no manifestations of poliomyelitis. It seems that the cord is no longer infective after 21 to 23 days.

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* This research was made possible through a grant received from the Trustees of the Banting Research Foundation.

[1] Flexner, S., *Science*, 1931, **74**, 520.

The infectivity of the cord declines rapidly for the first few days after complete paralysis and then more gradually until the end of the third week, when it is no longer infective. These results agree in a general way with those of Levaditi and Landsteiner,[2] who gave infection with cord obtained 4 days after paralysis, and with those of Flexner and Clarke,[3] Levaditi and Landsteiner,[2] Levaditi and Lépine,[4] and Landsteiner and Levaditi,[5] who found that at intervals longer than 3 weeks after paralysis, cord was no longer infective. Flexner and Clarke,[3] on the other hand, at times failed to infect with cord obtained a few days after paralysis, and Leiner and Wiesner[6] with cord obtained 6 days after paralysis.

A small series of animals were inoculated, intracutaneously, with some of these cords, in order to determine their immunizing power and to compare it with that obtained with acute cord. The first 2 received cord tissue in which virus was not demonstrated, one obtained 35 days after the height of paralysis, the other 23 days after paralysis and which had been washed as free as possible of antibody. The next 3 were given cord tissue obtained at 8, 4 and 2 days, respectively, after paralysis. The neutralizing power of the serums was determined in terms of minimal completely paralyzing doses and with the following results (Table II).

The immunizing power of spinal cord decreases markedly as the period after complete paralysis is prolonged and there is a relationship between the infectivity and immunizing power. Cord that proved non-infective failed to immunize, whereas cord which proved infective also immunized. That large quantities of cord, obtained 23 days after paralysis and washed as free as possible of antibody, failed to immunize, even though over neutralized virus-serum combinations give some immunity,[7] suggests an absence of

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[2] Levaditi, C., and Landsteiner, K., *Comp. Rend. Soc. de Biol.*, 1910, **68**, 311.

[3] Flexner, S., and Clarke, P. F., *J. Am. Med. Assn.*, 1911, 56, **8**, 585.

[4] Levaditi, C., and Lépine, P., *C. Rend. des Seances de la Soc. de Biol.*, 1931, **34**.

[5] Landsteiner, K., and Levaditi, C., *Annales de l'Inst. Pasteur*, 1910, **24**, 833.

[6] Leiner, C., and von Wiesner, R., *Wien. klin. Wchnschr.*, 1910, **23**, 91, 817.

[7] Brodie, M., *J. Exp. Med.*, 1932, **56**, 493.

TABLE 2.

			Neutralization Test 1				Neutralization Test 2				Neutralization Test 3			
Cord Specimen days after paralysis	No. Monkey	Gm. intra-cutaneously	Serum from Monkey No.	Serum cc.	MCP Doses Virus	Result	Serum from Monkey No.	Serum cc.	MCP Doses Virus	Result	Serum from Monkey No.	Serum cc.	MCP Doses Virus	Result
35	148	2	148	1	1	Par. 7 day								
23	204	1.5	204	0.9	1	Par. 8 day								
8	202	1.2	202	0.9	1	No paralysis	202	0.9	2	Par. 15 day				
4	294	1.2	294	0.9	2	" "	294	0.9	4	Par. 10 day				
2	299	1.2	299	0.9	16	" "	299	0.9	32	No paralysis	299	0.9	48	Par. 17 days
Height of Paralysis	233	1	233	0.9	16	" "	233	0.9	32	" "	233	0.9	48	" 17 "

CONTROLS

No. Monkey	MCP Doses Virus	Saline or Normal Monkey Serum cc.'s	Result
307	1	0.9 saline	Paralysed 6 days
283	2	0.9 "	" 6 "
296	1	0.9 "	" 6 "
348	1	0.9 serum	" 5 "
273	1	0.9 "	" 13 "

virus rather than its neutralization by antibody. The optimal time to collect cord tissue for both infection and immunization is at the height of paralysis.

6874

Active Immunization of Human Beings with Tetanus Toxoid.

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(Introduced by W. H. Park.)

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Apparently the first attempt at active immunization of man was made by Louis Bazy[1] working with Vallee. He gave iodized tetanus toxin obtained from Ramon at the Pasteur Institute to wounded soldiers. Three injections were given 5 days apart and 10 days after the last injection the men were bled and 1 cc. of the serum of each individual examined at the Pasteur Institute was found to contain from 10 to 100 "units" of tetanus antitoxin. In 1927 Ramon and Zoeller[2] used tetanus anatoxin, toxin treated with formaldehyde, and then incubated. They injected several hundred subjects. The greatest immunity was obtained by giving 0.5 cc. of anatoxin, 1 month later 1 cc. and 2 weeks later 1.5 cc. The immunity acquired was apparently so great that 1 cc. of the subject's serum neutralized 500 to 1,000 minimum lethal doses for a guinea pig. Twelve to 18 months later the immunity was still higher. After 1 to 2 years a single injection of anatoxin, the so-called "injection de rappel", resulted in a great increase in antitoxic power of the serum. 1 cc. of the serum neutralized 10,000 minimum lethal doses. The process of active immunization can not be hurried by shorter intervals between injections or large doses of anatoxin.

In May, 1930, we assembled 18 volunteers, 5 adults 18 years and over, and the rest children varying from 5 years up, who were bled and no trace of tetanus antitoxin found in their blood sera. At weekly intervals 3 doses of tetanus toxoid, made by treating the toxin with 0.4% formalin and incubating at 38° for 10 days, were injected intramuscularly. The injections were painful for about 5 minutes, but no general reactions occurred and no local ones beyond moderately sore arms. Four to 8 months later the individuals were bled again and the sera of 4, all children, contained definite amounts of tetanus antitoxin, 1 cc. of serum neutralizing 1 to 2 minimum lethal doses for guinea pigs. The rest contained traces of antitoxin, appreciable because the test guinea pigs survived the control pigs by several days.*

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[1] Bazy, L., *La Medecine*, 1926, **8**, 26.

[2] Ramon and Zoeller, *Comp. Rend. des Seances et Mem. de la Soc. de Biol.*, 1929, **100**, 92.

Six months later 5 of the subjects with no measurable amount of antitoxin were reinjected; 2 with 4 doses of 1 cc. of toxoid at weekly intervals; 2 with 3 injections of 1 cc. at weekly intervals, and 1 adult with 4 doses of 1 cc. at monthly intervals. The sera of the first 2, tested 7 and 8 months after the injections, neutralized 50 and 75 minimum lethal doses per cc.; the sera of the 2 cases receiving 3 doses each, tested 6 months later, in 1 case neutralized 25 minimum lethal doses and in the other still showed only a trace of antitoxin. The serum of the adult neutralized 40 minimum lethal doses per cc. Of the 7 cases showing a trace of antitoxin after the first series of injections and were not reinjected, 6 were retested 12 to 18 months after the first test. Three adults and 1 child still showed only a trace but 2 children had sera neutralizing 5 minimum lethal doses to the cc. Of the 4 children who had definite amounts after the first series of injections and were not reinjected, 3 were retested after 18 to 19 months and in all there was an increase in the antitoxin content. Just recently we have retested the blood of 13 of those originally injected 3 years ago. Of the 4 individuals who acquired immunity after 1 series of injections, 2 were retested and both still showed marked amounts of antitoxin. In addition 2 subjects who had only a trace of antitoxin after the first injections and were not reinjected, now after an interval of 3 years have appreciably increased the antitoxic contents of their sera.

Of the 6 individuals reinjected 18 months ago, 4 were recently retested. The child who developed no immunity after the second series of injections still has none. The other 3 still have large amounts of antitoxin. One adult lost some of his immunity and 1 child more than doubled the antitoxic content of the serum.

This series is very small. Several facts, however, stand out. It is possible by injection of tetanus toxoid in small doses at weekly intervals to create in the blood tetanus antitoxin which remains without further injections for at least 3 years. It is interesting to note that 1 child of 5 years was not immunized after 2 series of injections, but who, nevertheless, had become immune to diphtheria as judged by the Schick test, from 3 injections of diphtheria toxin antitoxin. The Department of Health has had similar experience with horses whose ability to make different kinds of antitoxin may vary.

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* The method of testing used was to combine each cc. of serum or fraction thereof with varying amounts of toxin. This was allowed to stand one-half hour and was then injected subcutaneously into 350 gm. guinea pigs and the results compared with the control guinea pigs.

6875

Histopathology of Central Nervous System of Mice Inoculated with Poliomyelitis Virus.

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From the Institute of Neurology, Northwestern University.

Harmon, Shaughnessy and Gordon[1] reviewed the contradictory results of former authors in inoculating rodents with the virus of poliomyelitis. They reported that after intraperitoneal or intracranial injection of the virus into mice the majority of them died sporadic deaths. The autopsy revealed gross lesions of lung consolidation, "but no microscopic sections were prepared from the tissues of mice".

The brain and spinal cords of 12 mice which had been injected with poliomyelitis virus by Dr. Nungester[2] together with those of 7 controls were cut serially and longitudinally and stained with cresylviolet. Four of the inoculated mice did not show any inflammation of the central nervous system. In 3 others mild inflammatory reactions in isolated segments of the dorsal and lumbar spinal cord were found in the form of perivascular leucocytic infiltration of one or 2 rows of cells and of a mild meningitis at the posterior surface in one case. In 3 other mice the leucocytic infiltration had spread into the posterior and anterior gray and was combined with hemorrhage into the anterior horns, mild increase in glia nuclei and meningitis along the spinal nerve roots. In 2 mice which had been injected with virus after passage through 3 gener-

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[1] Harmon, Paul H., Shaughnessy, Howard J., and Gordon, Francis B., *J. Prev. Med.*, 1930, **4**, 98.

[2] Nungester, W. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1128.

ations of mice and which had developed weakness of the hind legs a poliomyelitis was found which resembled more closely the histopathology found in poliomyelitis of macacus, though the inflammation was localized to a few segments only. There was a marked perivascular infiltration of polymorphonuclear and small mononuclear round cells around the central vessels, spreading diffusely into the gray matter and accompanied by hemorrhage into the anterior horns (Figs. 1 and 2). Besides there was a mild glia proliferation and the ganglion cells in these regions showed different degrees of degeneration, dust-like appearance or complete absence of Nissl bodies, pyknosis and beginning fragmentation of the nucleus with folding of its membrane. In some places beginning neuronophagia in the form of coffin-like arrangement of glia cells around a ganglion cell was present (Fig. 3). In one of 2 mice isolated diplococci were found within the leucocytic infiltration, measuring $1.2\mu\mu$ in longitudinal diameter and surrounded by a pale halo.

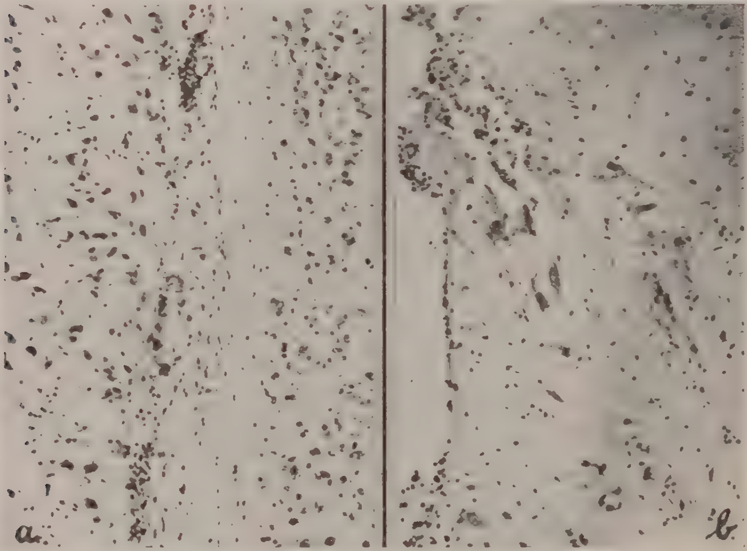

FIG. 1.

(a) Longitudinal section through spinal cord of mouse, Group III. Perivascular leucocytic infiltration around central vessel.

(b) Transverse section, second mouse of same group. Hemorrhage into anterior horn with diffuse leucocytic infiltration and ganglion cell degeneration. Original magnification 170 ×.

In none of these 12 mice an encephalitis was present. In the

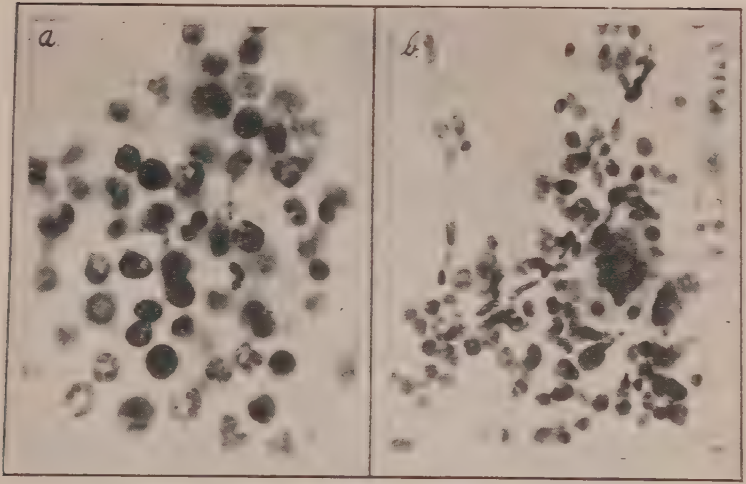

FIG. 2.

(a) Same case as Fig. 1 a. Infiltration of gray matter of spinal cord with polymorphonuclear leucocytes and mononuclear cells. Isolated diplococci surrounded by a pale halo are seen in the center of the field. Original magnification 1600 ×.

(b) Perivascular leucocytic infiltration in posterior horn with proliferation of glia nuclei. Original magnification 800 ×.

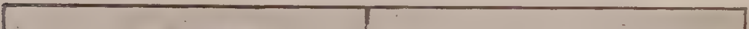

FIG. 3. Same case as Fig. 1 b.

(a) Anterior horn cell with beginning degeneration of cytoplasm and nucleus next to a focus of leucocytes infiltrating the anterior horn.

(b) Anterior horn cell with pale staining cytoplasm and pyknotic nucleus surrounded by an increased number of glia satellites (microglia?). Original magnification 1200 ×.

spinal cords of the controls isolated granulomas or foci of glia proliferation were detected; in 2 of them small isolated foci of leucocytes were seen along the spinal nerve roots. But in none of them a myelitis was found which could be compared with that described in the last 5 mice, which had been inoculated with the poliomyelitis virus.

6876

Effect of Injecting Pregnancy-Urine Extracts in Hypophysectomized Rats. I. The Male.*

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The injection of pregnancy urine (P.U.) in normal young rats causes an increase of the interstitial cells of the testes, an enlargement of the accessories and occasional injury to the tubules (Engle[1]). Collip *et al.*[2] reported that injections of placental extracts (which he considers physiologically similar to P.U.) in hypophysectomized male rats caused a hypertrophy of the interstitial tissue of the testes, prevented atrophy of the accessories, but *did not* prevent a loss in weight of the testes or degeneration of the tubules. Freud[3] reported that P.U. increased the size of the testes in hypophysectomized rats but gave no structural details.

We have injected several series of hypophysectomized male rats with Antuitrin S (P.U. extract) starting either immediately after hypophysectomy or after a 20-75 day post-operative period. For controls, either one testis was removed before injections were started or littermates were autopsied at that time. Untreated operated littermates were also autopsied at the end of the treatment period.

As evidence for the completeness of the pituitary ablation, we have: (a) daily weighings, (b) skeletal measurements taken at the

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

† National Research Fellow, Biological Sciences.

[1] Engle, E. T., *Anat. Rec.*, 1929, **43**, 187.

[2] Collip, J. B., and Selye, H., *Nature*, 1933, **131**, 56.

[3] Freud, J., *Deutsch. Med. Wochn.*, 1932, **58**, 974.

time of hypophysectomy and autopsy. (c) weights of adrenals and thyroids. (d) serial sections of the pituitary capsule. In no animals included in our data were fragments of definitely recognizable A.P. found.

Postponed injections of Antuitrin S in hypophysectomized male rats.

Hypsect.		Treatment begun			Injection		Autopsy	
Age	Wt.	After Hyps. Days	Wt.	L. test	Days	Total R. U.	R. test	Av. tubule area change
101	169	75	137	.139	10	100	.265	+15
43	71	34	81	.063	20	540	.188	+78
69	202	32	166	.252	21	1725	.436	+26
118	321	20	293	.513	27	675	.650	+60

Protocol. Immediate injections. Five littermates, males. Age at beginning of experiment, 111 days, weight, 267 gm.-302 gm. Weight of testes: reference control, 2.61 gm.; untreated hypophysectomized control autopsied 11 days after pituitary ablation, 2.02 gm.; treated hypophysectomized (27 R.U. per day Antuitrin S) for 12 days 3.2 gm., for 14 days, 3.19 gm. With long treatments as shown by other series the testes lose weight but less than untreated animals.

The injection of Antuitrin S has shown striking effects aside from the enlargement of the accessories and the hypertrophy of the interstitial tissue, in fact, the latter was less than we had anticipated from observations on similarly treated normal animals.‡ *There is a definite and pronounced stimulation of the seminiferous tubules. In the postponed injections, the testes approximately doubled their size, an enlargement which is only in part due to the interstitial-tissue hypertrophy. The tubules enlarge. Spermatids and even sperm may be formed even though none were present at the time treatment was instituted. In the injections begun at the time of hypophysectomy, regression in the weight of the testes was profoundly slowed, an enlargement even being found at times. The atrophy of the tubules was slowed and mature sperm continued to be present for a longer period than in untreated hypophysectomized littermates.* Though some tubules seemed to be injured by the P. U., it did have a stimulating effect on many other tubules. These results suggest that a beneficial effect on spermatogenesis might result from P. U. injection in cases of pituitary deficiency.

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‡ Thanks are extended to Dr. Oliver Kamm for a generous supply of this material.

6877

Effects of Injecting Pregnancy-Urine Extracts in Hypophysectomized Rats. II. The Female.*

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The results reported from the injection of P.U. into hypophysectomized female rats are conflicting. Reichert *et al.*[1] report no effect with such treatment; Wade *et al.*[2] agree, though reporting some luteinization and oestrus; Noguchi[3] reports thecal proliferation, follicular destruction and oestrus; Freud[4] reports similar effects as with A.P. treatment; Collip *et al.*[5-9] report thecal luteinization and induction of "a continuous oestrus from a shrinking ovary" in the adult, but not in the immature female.

We have injected over 40 mature and immature hypophysectomized female rats exclusive of controls with Antuitrin S (P.U. extract). Treatment was begun immediately or postponed 16-81 days after operation.

Animals injected immediately after operation, whether mature or immature, run a cornified (with an occasional mixed) type of vaginal smear.‡ In the postponed treatments, definite oestrus effects were invariably present as evidenced by larger uteri and oc-

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* Aided by a grant from the Committee for Research in Problems of Sex, the National Research Council.

† National Research Fellow, Biological Sciences.

[1] Reichert, F., Pencharz, R., Simpson, M., Meyer, K., and Evans, H. M., *Am. J. Physiol.*, **100**, 157.

[2] Wade, N. J., Katzman, P. A., and Jorgensen, M., *Proc. Am. Soc. Biol. Chem.*, 1933, xcvi.

[3] Noguchi, K., *Japan J. Med. Sc.*, 1931, Trans. IV, *Pharm.* **5**, 104.

[4] Freud, J., *Deutsch. Med. Wochn.*, 1932, **58**, 974.

[5] Collip, J. B., and Selye, H., *Nature*, 1933, **131**, 56.

[6] Selye, H., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 647.

[7] Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 665.

[8] Selye, H., Collip, J. B., and Thomson, D. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 780.

[9] Collip, J. B., Thomson, D. L., and Selye, H., *Proc. Am. Soc. Biol. Chem.*, 1933, xxxi.

‡ The animals were definitely immature as evidenced by the closure of the vagina and immature condition of the uterus and ovaries. We have not experimented upon rats as young as 22 days. These might fail to show an oestrus response.

casionally by the appearance of a mixed or cornified vaginal smear.§

The ovaries *always enlarge,* though with prolonged treatment they decrease again as in similarly-treated normal females. Ovaries of females treated immediately after operation showed hypertrophied interstitial cells and new corpora lutea, which were difficult to distinguish from those already existing in the case of the adult. The ovaries of females with postponed treatment showed the same marked interstitial cell hypertrophy and often corpora lutea formation. We are not prepared to state that these bodies arise only from thecal cells. Careful studies such as that of Corner on the corpus luteum of the sow will have to be made before the participation of the granulosa can be eliminated. In no case were follicles stimulated to grow. With continued treatment, and more rapidly with cessation of treatment, the ovaries diminished in size, leaving persistent corpora as discrete bodies surrounded by the involuted interstitial tissue.

POSTPONED INJECTIONS.

Immature Rats. Two littermates, 31 days old at hypophysectomy. No treatment for 16 days. One then autopsied. Weight of *both* ovaries, .0065 mg. Other injected for 10 days (25 R.U./day). *Left* ovary, .0102 gm., treated 19 days more, *right* ovary, .0081 gm. Two littermates, 39 days old at hypophysectomy. No treatment for 70 days. Left ovary, .0015 gm. Injected 6 days (25 R.U./day), right ovary, .0110 gm. The other injected 10 days, left ovary, .0095 gm. Treated 15 days more, right ovary, .0081 gm.

Mature Rats. Three littermates, 91 days old at hypophysectomy. No treatment for 78 days. Left ovary of each removed, weights, .0048, .0077, .0054 gm, respectively. All treated 10 days (10 R.U./day), weights of right ovaries, .0140, .0146, .0128 gm. respectively, percentage increases, of 192, 90, and 137.

IMMEDIATE INJECTIONS.

Immature Rats. Four littermates. 41 days old at hypophysectomy. One autopsied at beginning of experiment. Ovaries, .0123 gm. Two treated for 10 days (25 R.U./day). One autopsied at end of treatment, ovaries, .0510 gm. or 314% increase. Untreated operated sister, ovaries, .0083 gm. The other autopsied 11 days after treatment was stopped, ovaries, .0140 gm.

Mature Rats. Five littermates, 119 days old at hypophysectomy. One autopsied at beginning of experiment, ovaries, .0343 gm. One treated for 8 days (25 R.U./day), ovaries, .0485, or 41% over reference control. Another treated for 19 days, ovaries, .0460 gm., a 34% increase over the reference control. Another treated for 10 days, then a no-treatment period of 10 days, ovaries, .028 gm., an 18% decrease. The other, untreated, was autopsied after a 10 day postoperative period, ovaries, .0217 gm., a decrease of 37%.

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§ It seems clear that the oestrus was not due to the presence of oestrin, since 5 cc. (500 R.U.) of the Antuitrin S uniformly failed to induce oestrus in sensitized spayed female rats.

6878

Mating Reaction of Hypophysectomized Male Rats Treated with Pregnancy Urine Extracts.

PHILIP E. SMITH AND SAMUEL L. LEONARD.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Adult male rats are known to lose all libido immediately after hypophysectomy (Smith[1]), though matings for some time after castration have been reported. Also motile sperm have been demonstrated in the epididymis of hypophysectomized male rats no longer than 21 days after the operation (White[2]). Complete fertility is restored to hypophysectomized males by giving rat pituitary implants (Smith[1]).

Since it was observed that pregnancy urine extract (P.U.) slowed the degenerative changes in the testes of hypophysectomized rats (see previous article), matings were attempted at various times with oestrus females where treatment was begun immediately following the operation. Of 7 hypophysectomized males, 6 mated one or more times. In a total of 25 attempts at mating, 13 were positive and, furthermore, *11 of these were fertile, producing normal litters.* It was most interesting that of these fertile matings some took place 18, 20, 32, 34, and 47 days following hypophysectomy. It was not surprising because of the enlargement of the accessories that matings occurred, but the extended period of fertility was unexpected.

P.U. treatment restored the atrophied genital tract of hypophysectomized males to a degree when treatment was postponed following the operation. *That some hypophyseal factor is necessary and facilitates the restoring of the atrophied germinal epithelium is quite evident from our work, for the restoration secured by the injection of P.U. is not as complete as is the case with implants.* The relationship of P.U. to hypophyseal gonad stimulatory substances becomes even more obscure in the light of the findings reported in this series of papers.

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[1] Smith, P. E., *Am. J. Anat.*, 1930, **45**, 205.

[2] White, W. E., *Anat. Rec.*, 1932, **54**, 253.

6879

Difference Between Human Anterior Pituitary Extract and Prolan.*

SAMUEL L. LEONARD.†

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Numerous investigations in the last few years have indicated that the effect of administering anterior hypophyseal substances is different from that with the gonadotropic substances found in pregnancy urine. The tests are many: differential morphological changes in mice ovaries (Engle); limitation of the size of the immature rat ovary irrespective of dosage of prolan (Evans); negative response of prolan in birds (Riddle, Schockaert, Reiss); differential effect on hypophysectomized animals (Reichert, Kraul, Smith and Leonard, White and Leonard, Collip and Selye, Wade); and the relative ovulating doses in rabbits (Leonard). In all cases heretofore, the action of prolan was compared with that of the hypophysis of species other than human, namely, the cow and sheep. Recently, it was shown that the human hypophysis was unlike prolan when tested by the ovulation test method (Leonard[1]) in which the minimal ovulating dose in rabbits in terms of rat units equalled ¼ R.U., whereas with prolan it equalled 2 R.U. In this paper it will be reported that the human hypophysis is unlike prolan when tested by other methods.

There is a limit of response to increasing doses of prolan in ovarian enlargement in immature rats which is about 60 mg. (Evans). Twenty immature female rats were injected with doses from 1 mg. to 10 mg. equivalent of dried human anterior pituitary for 5 days. In all cases, the vaginas opened with marked oestrus effects and on the largest doses, the ovaries weighed up to 187 mg. Contrasting to this, 100 to 400 R.U. of prolan produced ovaries weighing only 45 mg. According to this test method, the action of the human hypophysis resembles the hypophyseal action of other species and is different from prolan.

Birds which appear to be non-responsive to pregnancy urine have been shown by others to be very responsive to hypophyseal extracts

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* Aided by a grant from the National Research Council, Committee on Problems of Sex, administered by Dr. P. E. Smith.

† National Research Fellow.

[1] Leonard, S. L., *Anat. Rec.*, 1933, **55**, 26.

of beef and sheep. Six immature white leghorn cockerels, 5 weeks old, were injected with doses of 20, 30, and 50 mg. of alkaline human A.P. extract for 10 days. These doses were not sufficient to increase the weight of the testes markedly, but on histological examination, the tubules were greatly enlarged and contained many mitotic figures. The response with the smaller doses was almost negligible, resembling the controls, but 30 to 50 mg. doses gave a very pronounced histological change. Testes of birds in the same group were unaffected following treatment with prolan.

Thus by 3 different test methods has the human hypophysis been found to be different in its physiological action from prolan.

The human pituitaries were obtained through the kindness of Dr. Kurzrok, of the Department of Obstetrics and Gynecology of the College of Physicians and Surgeons, and Mt. Sinai Hospital.

6880

Increased Skeletal Effects in A. P. Growth-Hormone Injections by Administration of Thyroid in Hypophysectomized, Thyro-Parathyroidectomized Rats.

PHILIP E. SMITH.

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University.

It has been shown (Smith[1]) and confirmed by a number of investigators that the injection of beef A.P. growth preparations, or the daily implantation of rat pituitaries caused a resumption of growth in rats dwarfed by hypophysectomy. Thyroid administration does not cause growth in the absence of the A.P. (Smith, Greenwood, and Foster[2]). It has been further shown (Flower and Evans[3]) that the A.P. growth hormone induces growth in rats dwarfed by thyroidectomy, a finding in harmony with the fact that A.P. extracts cause growth in hypophysectomized rats even though they may not repair the atrophied thyroids. It thus seems clear that of these 2 glands the A.P. only is essential for growth.

These findings, however, do not demonstrate that the thyroid may not normally aid the growth action of the pituitary or may not increase the effectiveness of preparations made from it. I have attempted to secure evidence upon this by giving growth hormone* with and without concurrent thyroid feeding in a series of rats in which both the hypophysis and thyroid (as well as the parathyroids) had been removed during the active growing period. All members of each series received the same dosage of growth hormone, one member receiving, in addition, thyroid. A dosage of thyroid was administered which did not prevent an increase in

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[1] Smith, P. E., *J. Am. Med. Assn.*, 1927, **88**, 158; *Am. J. Anat.*, 1930, **45**, 205.

[2] Smith, P. E., Greenwood, C. F., and Foster, G. L., *Am. J. Path.*, 1927, **3**, 669.

[3] Flower, C. F., and Evans, H. M., *Anat. Rec.*, 1925, **29**, 383.

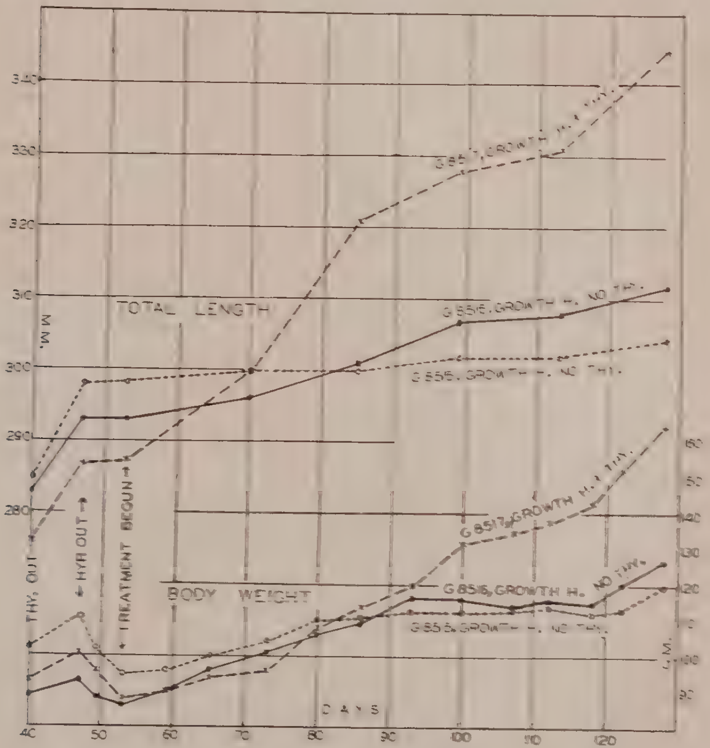

FIG. 1.

Curves showing total lengths and weekly averages of daily weighings of 3 littermate sisters which were hypophysectomized and thyroparathyroidectomized. All 3 received the same dosage of growth hormone, one of them, in addition, receiving thyroid by mouth. They were 40 days old at beginning of the experiment.

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* Growth hormone extracts of E. R. Squibb & Sons and Parke, Davis & Co. have been used. Appreciation is expressed to Drs. J. A. Morrell and O. P. Kamm for supplying these extracts.

weight (100 mg. powder 2-3 times per week). Four series of animals have been treated over a period of several weeks.

In each case the skeletal growth of the rats receiving thyroid in addition to the A.P. extract was distinctly above that of the rats receiving the extract only. In 3, the weight increases were greater also. The graph shows the characteristic effect.

These results suggest that the resumption of growth in thyroidectomized animals with thyroid feeding may not be due solely to a stimulation of the hypophysis but to a synergistic action of the thyroid. It also suggests that greater effects in human pituitary dwarfs might be secured by the concurrent administration of thyroid.

6881

Relative Amount of Mucin in Thick and in Thin Egg White.

EDMUND MC NALLY. (Introduced by Paul E. Howe.)

From the Poultry Physiology Laboratory, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Md.

The white of a hen's egg is readily separated into two portions, the thin white and the thick white. The yolk appears to be floating in thin white enclosed in a sac of thick white and this thick white is suspended in another (outer) layer of thin white.[1]

Previous studies of egg white have not demonstrated the nature of the difference between thick and thin white. In studying the proteins of egg white we have found that the thick portion of the white contains a much higher proportion of a protein possessing the properties of mucin than the thin white. This protein is soluble in an excess of mineral acids, insoluble in an excess of acetic acid, has a low nitrogen content (12.9%), and has the general physical properties of mucin. Examination of the eggs of some other birds indicates that this unequal distribution of mucin is not confined to the egg of the hen. Table I shows the mucin content of the eggs of the hen, pigeon and duck.

The whites of the eggs were separated on a screen as described by Holst and Almquist.[2] The thin white separated by this method con-

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[1] Almquist, H. J., and Lorenz, F. W., *Poultry Science*, 1933, **12**, 83.

[2] Holst, W. F., and Almquist, H. J., *Hilgardia*, 1931, **3**, 49.

TABLE I.

Source and character of egg white	No. samples	Mucin N in 100 gm. of sample Mg.	N in remainder of 100 gm. of sample Mg.	Total N in 100 gm. of sample Mg.
Hen egg	7			
Thick		94.5[1]	1,518	1613.5
Thin		10.4[2]	1,566	1576.4
Pigeon egg	2			
Thick		192.4	1,403	1595.4
Thin		40.5	1,444	1484.5
Duck egg	2			
Thick		263.6	1,553	1816.6
Thin		46.5	1,566	1612.5

[1] Standard deviation ± 28.65.
[2] Standard deviation ± 4.01.

tains both the outer and inner thin whites.[1] Tests of the inner thin white indicate that it may contain only minute quantities of mucin. It is possible that in the formation of eggs the mucin secretion and the serous secretion are poured out together and that the mucin particles coalesce, holding a certain amount of thin white, and that the volume of thick white varies with the changes in hydrogen-ion concentration.

Various authors, when discussing the preparation of the proteins of eggs, refer to the fact that they have dissolved egg white in salt solution or in water. We have found great difficulty in obtaining a "solution" of egg white in which particles of undissolved mucin are not present. It is probable that the mucin is seldom, if ever, obtained in solution in the sense in which most proteins are considered to be in solution.

A large proportion of thick white is of considerable economic importance since the quality of an egg is judged, in part, on the amount of thick white it possesses, as indicated in the opened egg by the unbroken envelop of thick white enclosing the inner thin white and yolk. Carbon dioxide has been shown[3] to have an effect in maintaining the quality of eggs, which probably depends largely upon the influence of the hydrogen-ion concentration on the physical properties of mucin. Mucin may likewise have a rôle in the regulation of the exchange of materials during incubation between the embryo and the several other parts of the egg and the surrounding atmosphere.

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[3] Sharp, P. F., and Powell, C. K., *Indus. and Engin. Chem.*, 1931, **23**, 196.

6882

Independence of Taste Organs with Respect to Their Nerve Fibers Demonstrated in Living Salamanders.

L. S. STONE.

From the Department of Anatomy, Yale University School of Medicine.

In these experiments the development of taste organs in embryos and larvae of *Ambiystoma punctatum* has been studied in the living specimen under high magnification with the aid of micro-aquaria. In one series part of the anlagen of the tongue from the endodermal floor of the pharyngeal cavity was transplanted to the side of the body and oriented so that the smooth glistening surface of the endoderm was exposed to the outside. The donor and recipient in a single experiment were usually similar in age. These grafts were from donors ranging in ages from the tail-bud to the early swimming reaction stages and long before the first appearance of taste organs normally takes place. This series contains donors in which either no cranial nerves had yet formed or no nerve fibers had reached the region from which the graft was taken.

They showed that normal taste organs appeared in the transplants at the normal time (about stage 40 of Harrison series, several days before the mouth is open) and that they increased in number and persisted up to and through metamorphosis as the grafted tongue grew in size. The increase in the number of organs is by a process of budding brought about by mitosis and often a migration of the peripheral supporting cells. This is similar to that described by the author[1, 2] in the case of accessory lateral-line organs.

These results prove that the taste organs in this form develop *in situ* and are independent of any specific influence on the part of gustatory nerves to initiate their formation. The results also strongly suggest that the initiation of their development is entirely independent of any reaction on the part of nerve fibers which might grow in from the new environment. Whether later their maintenance is dependent upon an association with a nerve supply of a new source is not proved.

A second series of experiments were made on older stages. In these cases the grafts of the tongue anlagen, placed upon the side of

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[1] Stone, L. S., *Anat. Rec.*, 1933, **55**, 38.

[2] Stone, L. S., *J. Comp. Neur.*, 1933, **57**, in press.

the body, were taken from donors just before, during and after taste organs normally appear. The operation had no effect upon the appearance of the organs. They were normal in structure and rapidly increased in number as in the first series of experiments. These grafts were followed for weeks throughout the larval period of development and finally through metamorphosis.

In a third series of experiments a still older group of specimens was taken. As in the other series the donors and recipients of grafts were similar in age and length. They include stages from the time of the beginning of feeding (Harrison stages 45-46, about 15 mm. in length), up to 33 mm. larvae. In these a small portion of the tip of the tongue with taste organs was grafted to the side of the body and on the tail fin. The organs in these cases not only did not disintegrate when isolated from their normal nerve supply but continued to give off bud organs and by this process rapidly increased the number of new structures. Grafts containing a few organs, in some cases 1 or 2, were conveniently placed upon the transparent tail fin. In such a transplant a complete new bud-organ is seen to form within 3 days after operation. The new organ may or may not start a new bud formation immediately. The detail can be followed under high power.

In the *A. punctatum* larvae it is evident from the second and third series of experiments that an immediate isolation from the nerve supply does not interfere with any developmental stage in which the taste organs may be. It also does not cause a disintegration of organs already formed or in the process of budding. The rapidity of the onset of the normal process in the formation of new organs following operation seems to suggest that its initiation is independent of any intimate relation with a new and strange nerve supply.

The results of these 3 series of experiments leads one to the conclusion that the suggestions of a hormonal-like influence of the peripheral nerves[3-7] in maintaining the structure of the taste organs and in initiating[8] their development can not be applied to the embryos and larvae of *Amblystoma punctatum*.

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[3] May, R. M., *J. Exp. Zool.*, 1925, **42**, 371.

[4] Olmstead, J. M. D., *J. Comp. Neur.*, 1920, **31**, 465.

[5] Olmstead, J. M. D., *J. Exp. Zool.*, 1920, **31**, 367.

[6] Parker, G. H., 1922, Philadelphia and London.

[7] Torrey, T. W., *Proc. Nat. Acad. Sc.*, 1922, **17**, 591.

[8] Landacre, F. L., *J. Comp. Neur.*, 1907, **17**, 1.

6883

Developmental Changes in Primary Lateral-Line Organs Studied in Living Larvae of Anurans and Urodeles.

L. S. STONE.

From the Department of Anatomy, Yale University School of Medicine.

In amphibian embryos the migrating lateral-line primordia lay down a series of primary organs which can be observed in the living and vital-stained condition.[1, 2] Since the mid body line extends down the tail in rather transparent tissue such organs are favorable for study in the living specimen when observed under high magnification in micro-aquaria. These data concerning the developmental changes in primary tail organs of normal larvae provide a basis for further observations consequent to the cutting of the lateral-line nerve.

In *Amblystoma punctatum* larvae the large melanophores often obscure the view of many lateral-line organs and their nerves. This condition was easily avoided by feeding specimens an exclusive diet of enchytraea which, for some unknown reason, contracts the dark pigment cells. The animals become pale yellow in color. In *Hyla crucifer* tadpoles pigment presents no serious difficulties. In contrast to the normal pigmented larvae of *Rana palustris* it was found that hypophysectomized specimens gave as clear a picture as Hyla.

In *Amblystoma punctatum* the tail organs begin abruptly at a level above the anus. In many larvae studied at the feeding stage (Harrison stage 46, 15-16 mm.) such a line on the tail is about 18 in number, extremes being 13 to 20. As in other lines the number on the right side is usually not equal to that on the left.

From primary organs accessory ones are formed by budding from supporting cells.[1, 2] Shortly after the feeding stage begins the terminal organ may give off a bud posteriorly and thus form a chain of 1 to 3 new organs. However, normal isolated animals often do not show an immediate response by budding of the terminal organ until later. In larvae around 30 mm. this organ, which then usually lies some distance from the tip of the tail, very often begins to show budding and the formation of a few organs in the posterior direction. Although in some normal larvae this does not

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[1] Stone, L. S., *Anat. Rec.*, 1933, **55**, 38.

[2] Stone, L. S., *J. Comp. Neur.*, 1933, **57**, in press.

occur, when it does it seems to take place more often around this period.

When larvae reach 20 to 24 mm. a sudden budding process almost always involves the dorsal and ventral poles of primary tail organs. Often a bud-organ is dorsoposterior or ventroposterior in its point of origin. In a cluster of the usual 3 organs, 1 primary and 2 bud-organs, the accessory ones are rarely confined to one pole. The more proximal primary organs on the tail usually bud first, for this process spreads from the body region of the line. The budding rapidly includes more posterior organs until at 30 mm. it may involve all but the last 7 or 8 organs. At this time, by rapid budding, the original number of tail organs may nearly double and by 40-45 mm. the number greatly increases because all but the terminal or last 2 or 3 organs are included. Although bud-organs may shift slightly from their source of origin, usually in the posterior direction, the tendency is for organs to remain in groups of 3 and sometimes 2. Four may occasionally be found.

From 40 to 50 mm. there seems to be normally no new bud-organ formation. During this late larval period the organs are gradually covered by a layer of flattened surface epidermal cells—a condition which anticipates that relation of these cells to the organ in the metamorphosed stage in Amblystoma.

In *Rana palustris* and *Hyla crucifer* larvae the many primary organs on the tail exhibit budding much more rapidly in contrast to that of *A. punctatum*. The terminal organ seems to be continually forming new bud organs in the posterior direction up to late larval life. The general conditions are the same in Rana and Hyla. In Rana from 10 to 15 mm. many primary organs at both anterior and posterior poles give off bud-organs, thus increasing rapidly the original number of organs in a single line. At about 13 mm. budding begins at the dorsal or ventral poles of the organs, and as the animal increases in length this spreads posteriorly. By 20 to 25 mm. most of the organs are involved and groups of 2 are formed. From this another rapid bud formation takes place in either the dorsal or ventral direction or both so that a group may contain as many as 6 or more organs. They may be grouped in irregular clusters due to the method of budding, but are nearly always in a linear arrangement extending in the dorso-ventral direction.

6884

Observations Following Distension of the Gallbladder and Common Duct in Man.

ROBERT ZOLLINGER. (Introduced by Elliott C. Cutler.)

From the Surgical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

The susceptibility of the gallbladder and common duct to overdistension from various causes makes them a common source of visceral and referred pain. The location of pain in gallbladder disorders varies and there is nothing in its character to differentiate it from pain resulting from a change in the state of the common duct. Patients with biliary disease commonly complain of chronic indigestion and, in addition, their pain may be referred to the interscapular and infrascapular regions, right upper quadrant, epigastrium and occasionally to the left side. Various theories have been proposed to explain the mechanism of this "referred" visceral pain.[1, 2]

Our observations were made on six women operated upon for gallbladder disease. Their complaints varied from chronic indigestion to typical, as well as bizarre, radiation of the pain. Chronic cholecystitis and choleliathiasis were found in every case at operation.

Method. In 4 patients the gallbladder was exposed and the stones removed after a short gas-oxygen anesthesia. A sterile rubber balloon, carried on a sterile hollow metal rod, was inserted into the gallbladder. The rod was connected to a blood pressure apparatus through which the balloon could be inflated and the amount of pressure recorded. The fundus of the gallbladder was then sealed around the rod by several sutures and the organ replaced in the abdomen. The edges of the wound were held in approximation and all gauze and retractors removed from its margins to avoid additional painful stimuli. The distension was carried out after the patient had recovered sufficiently from the short anesthesia to answer questions intelligently. In one patient the observations were made following exposure of the gallbladder under local infiltration anesthesia of the right upper quadrant. In a second patient the epigastrium was infiltrated with novocaine after distension of the gallbladder following gas-oxygen anesthesia. The common duct

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[1] Mackenzie, J., Symptoms and Their Interpretation. London, 4th edition, 1920.

[2] Morley, John, Abdominal Pain. William Wood and Co., New York, 1931.

was distended in 2 of the 6 patients by the same method and the resultant signs and symptoms similarly recorded.

Our observations seem to indicate that: 1. Distension of the *gallbladder* gives rise to deep epigastric discomfort more severe but similar to the attacks of indigestion in gallbladder disease. 2. The discomfort is not referred to the gallbladder region. 3. Distension of the gallbladder does not cause nausea and vomiting. 4. Distension of the *common duct* is much more painful than distension of the gallbladder and in addition is accompanied by nausea and vomiting. 5. Objectively and subjectively the patients have respiratory difficulty when the gallbladder or common duct is distended, especially inspiratory difficulty. 6. It was found impossible to reproduce the usual referred pain in the back, infrascapular region or the right upper quadrant. 7. The pain was not altered in one case in which the right upper quadrant was novocainized. 8. Distension of the gallbladder, producing contact with the parietal peritoneum, gave localized pain. This was markedly relieved by infiltration of the overlying abdominal wall with novocaine.

Since referred pain could not be produced by mechanical distension, weight is given to the argument that referred somatic pain and tenderness are more likely to result from an inflammatory lesion caused by a "peritoneocutaneous" reflex instead of a "viscerocutaneous" reflex. It is indicated that surgically it might be well to explore the common duct in those cases in which vomiting has been a prominent symptom, regardless of the history of jaundice or size of the common duct.

6885

Pathogenesis of Trichinous Myocarditis.

GLEN L. DUNLAP AND CARL V. WELLER.

From the Department of Pathology, University of Michigan.

Several authors have recognized and described the histopathology of the parenchymatous lesions and accompanying myocarditis which are seen in the hearts of human cases of trichinosis dying in the fourth to eighth week of the disease. The importance of these lesions in the production of the circulatory failure, which is the cause of death in these cases, has been emphasized.[1] All who have had opportunity to study such material have noted the absence of encysted larvae in the heart muscle even though the skeletal muscles showed at the same time very many encysting and encysted organisms. Almost all of those who have searched sections prepared from such hearts have failed to find larvae present at all. Only Zenker[2] and Frothingham[3] saw them in the human heart, but Graham,[4] working with rats, described lesions similar to those in human cases and in addition found embryos associated with the inflammatory foci. This complete absence of encysted forms with but meager evidence even of the presence of larvae, has led to uncertainty as to the pathogenesis. Simmonds[5] concluded that the myocarditis was due to a toxin produced by the trichinae and brought to the heart in the circulating blood.

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[1] Weller, C. V., and Shaw, M., *Trans. Assn. Am. Physicians*, 1932, **47**, 41.

In the present study, series of white rats were fed, in approximately known numbers, the larvae digested (McCoy[6]) from trichinous meat. Animals were killed at appropriate intervals and both fresh, teased preparations and stained sections were made from their tissues. The myocardium showed alterative and exudative lesions in all respects comparable to those found in human hearts. Trichina embryos were found in these foci as early as 5 days after feeding and for some time thereafter. In rats examined after active migration of larvae had ceased, the myocardium showed no reaction, although encystment of larvae in skeletal muscle was occurring to a marked degree. If the myocarditis were of toxic origin it should not have subsided during this stage. Thus by 2 lines of evidence it appears that it is the presence of the larvae in the myocardium and their active migration and not a blood-borne toxic substance which produces the characteristic myocarditis. It is not clear what are the factors, probably inherent in the heart muscle, which prevent encystment.

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[2] Zenker, F. A., *Arch. f. path. Anat. u. Physiol.*, 1860, **18**, 561.

[3] Frothingham, C., *J. Med. Research*, 1906, **15**, 483.

[4] Graham, J. Y., *Arch. f. mikr. Anat.*, 1897, **50**, 219.

[5] Simmonds, M., *Centralbl. f. allg. Path. u. path. Anat.*, 1919, **30**, 1.

[6] McCoy, O. R., *Am. J. Hyg.*, 1931, **14**, 484.

6886

Reduction of Blood Volume Following Bilateral Oophorectomy.

MAE FRIEDLANDER, NORMAN LASKEY AND SAMUEL SILBERT.
(Introduced by Robert T. Frank.)

From the Out-Patient Department and Laboratories of The Mount Sinai Hospital, New York City.

The present work was stimulated by the striking changes in blood volume found in patients with thrombo-angiitis obliterans by Silbert, Kornzweig and Friedlander.[1] The fact that this condition rarely occurs in women suggested that some physiological difference accounts for their immunity. It was natural to assume that the ovaries might be responsible for this difference. The occurrence of repeated attacks of superficial phlebitis in the lower extremities during the years following the removal of the ovaries has been observed by us and by others. Some alteration in the blood may play a rôle in producing this manifestation. Although several investigators have reported changes in the chemistry of the blood after bilateral oophorectomy, none have included a simultaneous study of the blood volume.[2, 3]

The present report includes a presentation of data on blood volume, viscosity, cholesterol and fibrinogen in 25 patients who have had surgical removal of both ovaries and the uterus, and a miscellaneous group of 27 patients comprised as follows:

Natural menopause	9
Artificial menopause produced by radiotherapy	8
Recurrent amenorrhea	1
Single oophorectomy	2
Miscellaneous operative cases	6
Pre-operative case	1

Blood volumes on about 50 normal individuals gave an average figure of 80 to 85 cc. per kilo. The same results were obtained by other investigators.

The dye method of Lampe modified by Rosenthal was employed, as described by Silbert, Kornzweig and Friedlander.[1] The cholesterol determinations were made by the method of Bloor and the fibrinogen tests by the new Wu method. The Hess apparatus was used in estimating the viscosity.

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[1] Silbert, S., Kornzweig, A. L., and Friedlander, M., *Arch. Int. Med.*, 1930, **45**, 948.

[2] Geist, S. H., and Goldberger, M. A., *Am. J. Obstet. and Gyn.*, 1926, **12**, 2.

[3] Bailey, H. C., *Am. J. Obstet. and Gyn.*, 1921, **2**, 1.

The average blood volume in the bilateral oophorectomy cases shows a reduction of about 25% from the normal figure. This change becomes more striking after the first few months. Coincident with this there is an elevation in the cholesterol and fibrinogen content of the plasma, and an increase in the viscosity. Since the cholesterol and viscosity figures are easily altered by physiological changes, such figures should not be given too much weight. However, in our cases there is a consistent tendency for these figures to be higher and they are supported by the diminished blood volumes.

In only one patient who had a bilateral oophorectomy was a normal blood volume obtained. Here, there was definite clinical evidence of hyperthyroidism and the basal metabolism was +47%. The normal blood volume in this case is probably due to the increased thyroid activity. Silbert and Friedlander[4] have pointed out that restoration of normal blood volume in thrombo-angiitis obliterans cases can be brought about by the administration of thyroid extract.

Corresponding studies of the miscellaneous control group reveal blood volume figures which are within normal limits. This group includes 17 patients with normal or artificial menopause. It would appear, therefore, that some substance capable of influencing blood volume is produced by the ovary, even after other physiological functions have ceased.

6887

Individual Feather Succession in the Hybrid Capon.*

MARY JUHN. (Introduced by F. R. Lillie.)

From the Whitman Laboratory of Experimental Zoology, the University of Chicago.

The plumage of the F-1 males resulting from a cross in either direction between Barred Rocks and Brown Leghorns in general

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[4] Silbert, S., and Friedlander, M., *J. Am. Med. Assn.*, 1931, **97**, 17.

* The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by F. R. Lillie.

resembles that of the Barred Rock though the pattern is not strictly identical.

Spaced among the barred plumage are feathers, the so-called exceptional feathers, which may be completely identical with those of the Brown Leghorn male, or, while exhibiting both barred and leghorn characteristics in general, yet show a number of traits foreign to either parent. The occurrence of these exceptional feathers is practically entirely confined to the plumage regions in which barbules are restricted to the vicinity of the shaft and to the basal feather sections, the saddle, hackle, head and minor coverts.

The greater number of exceptional feathers possess a barred tip and leghorn base; the barred region may occupy one vane-half, or the margin only of the feather, in which case the transition takes place within individual barbs, and yet other variations.

Feathers similar to these have been described in hybrids resulting from a cross between Plymouth Rocks and Orloffs by Serebrovsky,[1] by Hertwig and Rittershaus[2] from a cross between Barred Rocks and Goldlack, and from a cross between Plymouth Rocks and Faverolles by Kuhn.[3]

Serebrovsky attributes the origin of the exceptional feathers to the loss of one sex-chromosome carrying the factors for barring in development; Hertwig and Rittershaus to the frequent loss of the part of the X-chromosome containing the factor which inhibits black and white regions of barred feathers. Both Kuhn and Hertwig and Rittershaus state that exceptional follicles always produce exceptional feathers identical in pattern with the exceptional feathers plucked.

In this laboratory the methods described in earlier papers for the identification of individual follicles were applied to 10 adult hybrid capons from the cross described above: a total of 186 individual feathers severally identified were followed through one regeneration. Of this number 102 barred feathers were replaced by barred; 26 barred by exceptional; 46 exceptional by barred and 8 exceptional by exceptional. No regeneration of exceptional feathers identical to the original were found.

Where the observations were continued to 2 successive regenerations, of the possible combinations 7 were found: barred × barred × barred 44; barred × barred × exceptional 11; barred × exceptional × barred 7; barred × exceptional × exceptional 4; exceptional × barred × barred 20.

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[1] Serebrovsky, A. S., *J. Genetics*, 1926, **16**, 35.

[2] Hertwig, Paula, and Rittershaus, Tine, *Arch für Geflügelkunde*, 1929, **3**, 65.

[3] Kuhn, Otto, *Z. für Züchtungskunde*, 1928, **3**, 615.

These findings do not support the genetic interpretations advanced, which, incidentally, are untenable from an embryological point of view.

It therefore appears in greater harmony with the observations to consider the potentialities for both barring and leghorn as continually present in the plumage of the hybrid. The exteriorization of one or other character as well as the intermediate forms may be considered to rest upon the interaction of the genetic factors for these characters with variable morphogenetic factors such as rate of growth which has been shown to be effective in the production of variations in pattern in the feather[4] and in the entire plumage.[5]

Hence in identical physiological milieu regeneration of identical exceptional feathers may indeed be encountered. It also follows that determination of the plumage pattern of the hybrid should prove amenable to agents acting through the cytoplasm such as temperature, etc., and while considerable difficulties are to be expected, experiments along these lines are now under way.

6888

Experiments in Endobronchial Stenosis.

CORNELIUS B. WOOD. (Introduced by Elliott C. Cutler.)

From the Laboratory of Surgical Research, Harvard Medical School.

The production of bronchial stenosis by the simple method of the endobronchial application of a caustic has given rise to considerable experimental work. Adams and Livingstone painted the orifice of a bronchus with a 35% silver nitrate solution, which resulted in complete stenosis of that bronchus in a high percentage of attempts. This demanded repetition in view of the many possibilities for the application of such a method in clinical conditions.

Following the technique of Adams and Livingstone[1] we have failed to produce complete stenosis in either dogs or monkeys in experiments during the last 1½ years. Either we produced so great a destruction that rupture of the bronchus or a serious pneumonitis distal to the bronchus occurred, or stenosis failed to result. Stenosis with complete collapse of the lobe distal to this occurred in but a small percentage of our animals.

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[4] Lillie, Frank R., and Juhn, Mary, *Physiol. Zool.*, 1932, **5**, 124.

[5] Juhn, Mary, and Gustavson, R. G., *J. Exp. Zool.*, 1930, **56**, 31.

[1] Adams, W. E., and Livingstone, H. M., *Ann. Surg.*, 1932, **95**, 106.

The desirability of the method led to a search for other solutions which might be more efficacious. To produce a permanent bronchial occlusion by a safe method, the substance should act slowly and over a long period of time. It must penetrate deeply and produce a peribronchial fibrosis with little damage to the mucosa of the bronchus or to the lung distal to the application. If it be used on the bronchus to a lobe, the seat of an inflammatory process, there should be ideally no denudation of the epithelium of the bronchus.

Concentrated solutions of the acridine dyes were tested for this purpose, since it is well known that when these dyes are used for urethral irrigation they produce considerable periurethral fibrosis. Our experiments with 37 dogs show that a 25% aqueous solution of acid acriflavine applied to a bronchus by the bronchoscopic route produces with great regularity in from 3 to 4 weeks an occlusion of that bronchus. One end of a stiff wire is wrapped with cotton and this is moistened with the solution. The cotton applicator should be large enough to fit the lumen of the bronchus to be treated snugly, so that the acriflavine may have contact with the entire surface of the wall of the bronchus. Care must be observed to wipe any excess from the cotton applicator before its insertion. The applicator is left firmly in place in the bronchus for 6 to 8, and occasionally 10 minutes.

Following treatment, the dye quickly penetrates to the peribronchial tissues. If such a bronchus is examined one to 2 hours later the intense yellow staining can be seen in these tissues. The epithelium and outer layers of the submucosa are destroyed by the drug, but within one to 3 days a firmly adherent mucoid plug forms which completely encases and protects this denuded area. A temporary atelectasis is thus formed in the lung distal to the plug.

This mucoid plug remains in place for 8 to 10 days, at which time the rapidly regenerating epithelium has grown over the submucosa, separating it from the mucoid plug. The loosened cast is then coughed out, and the lung distal becomes air-containing. Thus at no time is the damaged wall exposed to possibilities of infection.

Complete collapse of the middle and accessory lobes occurred with a single application. In the case of the upper and lower lobes at most 2, and in rare cases, 3 applications were necessary, largely because of the numerous, partially hidden orifices to these lobes. The peribronchial connective tissues have become fibrosed within 3 to 4 weeks and they contract, compressing and buckling the bronchial cartilages, to produce a partial collapse of the lumen of the bronchus. Proliferation of fibrous and granulation tissue from the submucosa further compresses the mucosa and occludes the lumen of the bronchus.

The procedure described is quite safe and surprisingly efficient. It is so benign that the bronchi of an entire lung may be safely treated at one bronchoscopy. Grossly, at bronchoscopy, there can be seen no ulcerations on the mucosa. There have occurred no pulmonary hemorrhages, and sections show little or no damage to the blood vessels. The dogs following bronchoscopy, even after the bronchi of an entire lung have been treated, appear in good condition, and it is rare that they run one to 2 degrees of temperature. They eat well and, except for a hacking cough, appear entirely normal. In our first 12 animals, when the technical application had not been entirely determined, 4 deaths occurred, 2 from pushing the applicator through the bronchus, and 2 from overcauterization. In the last 25 animals 100% were given satisfactory and complete stenoses.

It would appear from such observations on these dogs, that a simple and efficient method for the production of bronchial stenosis has been obtained.

6889

Ascorbic Acid from Iris and Other Plants by a Simplified Method.*

EMIL J. BAUMANN AND NANNETTE METZGER.

From the Laboratory Division, Montefiore Hospital, New York City.

We have had great difficulty in obtaining more than a few milligrams of the antiscorbutic vitamin, ascorbic acid, from either plant sources or suprarenal glands by the published methods of Szent-Györgyi[1] or of Waugh and King[2] and Svirbely and King.[3] Large

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Szent-Györgyi, A., *Biochem. J.*, 1928, **22**, 1387.

[2] Waugh, W. A., and King, C. G., *J. Biol. Chem.*, 1932, **97**, 325.

[3] Svirbely, J. L., and King, C. G., *J. Biol. Chem.*, 1931, **94**, 483.

and uncontrolled losses occur at many places in both processes as published. Svirbely and Szent-Györgyi[4] have just reported a somewhat modified procedure, using the Hungarian sweet pepper from which paprika is made, which contains considerably more vitamin C than we have obtained from the American sweet pepper—another variety of *Capsicum annuum.* We have studied the causes of these losses with a view to developing a more certain method of preparation. A survey of common plants was also made to find richer sources of the vitamin. Some of these results have been published by Marine, Baumann and Webster.[5] Leaves of German iris *(Iris germanica)* are by far the most satisfactory material from which to extract vitamin C, both because the vitamin is present in high concentration and because there are fewer substances in this plant that interfere with its separation. Most plants have a relatively high concentration of ascorbic acid in the young actively growing portions but on maturation, the vitamin rapidly disappears. In German iris leaves, however, the concentration of the vitamin, which is 600 mg. per 100 gm. of fresh leaf in early spring, remains as high as 250 or 300 mg. per 100 gm. in late summer, and this plant offers a very rich source during the entire growing season. Skunk cabbage *(Symplocarpus foetidus)* gathered during April and May and sweet green peppers as purchased from January to May both contained between 100 and 200 mg. per 100 gm.

We have developed a comparatively simple method for preparing 5 to 20 gm. of ascorbic acid at a time from 5 to 10 kg. of these plants. Several of the steps developed by King and his associates in extracting crystalline vitamin C from lemons have been utilized. We are also indebted to Nelson,[6] who showed that in the presence of traces of copper, ascorbic acid is rapidly oxidized, and to Marine,[7] who finds ferric iron acts similarly though it is only about half as active as copper. The ascorbic acid is isolated as the monoacetone derivative described by Vargha.[8]

From iris leaves, about 70-75% of the reducing substances originally present was obtained as the crystalline acetone derivative of ascorbic acid. From skunk cabbage leaves and from sweet green peppers, the yields were 35% and 60% respectively. From the mother liquors, which can be combined, considerable additional amounts have been readily obtained on further purification.

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[4] Svirbely, J. L., and Szent-Györgyi, A., *Biochem. J.*, 1933, **27**, 279.

[5] Marine, D., Baumann, E. J., and Webster, B., *J. Biol. Chem.*, 1930, **89**, 213.

[6] Nelson, E. K., *Science*, 1932, **76**, 345.

[7] Marine, D., unpublished data.

[8] Vargha, L. v., *Nature*, 1932, **130**, 847.

The specific instructions which follow are simpler, more readily followed and better adapted to the apparatus ordinarily found in most laboratories than those published hitherto.

Place 5 kg. of iris leaves in cheese cloth or scrim press cloth and steam for 30 minutes in an instrument sterilizer. Hash and press out juice in a wine press. The press cake may be reextracted with acidulated water or methyl alcohol over night and an additional 10-15% may be obtained in this extract, though ordinarily this is not worth the extra effort. Concentrate the press juice *in vacuo* at a water bath temperature of 40-50°C., with a capillary through which CO_2 is passed, to 1/5 its volume. Add 3-4 volumes methanol gradually. Filter on a large Buchner, wash the gelatinous precipitate once on the filter and then again by rubbing up in a mortar with a large volume of 75% methanol. Concentrate the filtrate and washings *in vacuo* to 400-500 cc. Extract 3-5 times with 100-125 cc. butanol in a separatory funnel. If separation does not take place reasonably quickly, the fluid may be centrifuged in 250 cc. cups and the aqueous layer aspirated off. Extract the butanol solution of fats and pigment once or twice with 50 cc. water, combine the aqueous extracts and chill to 0-5°C. Sufficient hot saturated solution of neutral lead acetate is added to make the concentration 25-30%. Keep chilled and gradually add 15% ammonium hydroxide to a pH of 7.6-7.8. Centrifuge off the precipitate and wash once in the cups with a large volume of water. Rub the precipitate up to a thick paste with cold water, transfer to a liter beaker and add cold 15% HCl to a pH of 2 (pink with thymol blue). Filter on Buchner, wash the PbCl in a mortar with cold water acidulated with HCl. Concentrate filtrate and washings *in vacuo* with a capillary through which CO_2 passes, to a thick syrup. The fluid darkens but apparently no loss of the vitamin takes place. Add 200 cc. of methanol, evacuate flask and heat under hot water (50-70°C.) until the gum is dissolved. Cool and gradually add 3-5 volumes of acetone, until precipitation is nearly complete. Centrifuge off gummy precipitate or oil (most remains in flask). Dissolve the gum in the centrifuge bottles in 50 cc. methanol and add to the main part of the gum in the flask which is then also dissolved as before. Then add 5 volumes of acetone gradually. Centrifuge again and treat the gum a third time with methanol and acetone. Concentrate the combined alcohol-acetone extracts *in vacuo* to a thick syrup.

Dissolve the syrup in an evacuated flask in 50 cc. of dry methanol, cool and add slowly with shaking about 10 volumes dry acetone and finally 500 cc. of pentane. Centrifuge off. Dissolve the oily residue in 50 cc. dry methanol, add 10 volumes of dry acetone and 10 volumes of pentane and centrifuge again. The oily residue is treated in this way 4-6 times. Concentrate the combined acetone pentane extracts to a thick syrup *in vacuo*. Add 30-40 cc. dry acetone. Evacuate the flask and warm in hot water until the syrup dissolves. The evacuated flask is kept chilled to 0-5°C. for about 2 days. A crop of crystals is then filtered off and washed with cold acetone until nearly white. 9.3 gm. of the acetone derivative were obtained at this point.

Dilute the mother liquor to 200-300 cc. with dry acetone and add 1½-2 volumes of pentane. Centrifuge off the oily residue, dissolve in 5 cc. methanol and treat again with acetone and pentane until nearly all of the vitamin has been extracted. Concentrate extracts *in vacuo* to a syrup, add 25 cc. of dry acetone and store in an ice box in an evacuated flask over night when another crop of crystals separates. 6.6 gm. were obtained, making a total of 15.9 gm. of the acetone derivative from 5 kg. of iris leaves. More can be separated from this mother liquor.

The amount of vitamin present at each stage can be conveniently followed by titrating small aliquots (0.5-2.0 cc.) with 0.01 N iodine solution in acid solution, with starch as an indicator.

Press juices from skunk cabbage leaves and green peppers have been treated similarly but an additional treatment with methyl alcohol and acetone is necessary in the first part of the process, before pentane can be used, and an additional fractional separation with acetone and pentane may also be needed.

Purification of Reagents. Synthetic methanol is distilled from alkali. Acetone is refluxed for half an hour with $KMnO_4$ and then distilled through a fractionating column. Acetone and methanol are dried sufficiently with anhydrous Na_2SO_4 for use in the latter part of this process and then distilled from alkali in the case of methanol or refluxed with $KMnO_4$ and distilled in the case of acetone. Normal butanol is used as purchased. Commercial pentane (B.P. 30-35°C.) is shaken with concentrated H_2SO_4 in a separatory funnel and distilled. Lead acetate (analytical grade) is recrystallized twice from water to remove traces of copper.

The crude acetone ascorbic acid from iris melted at 219.5°C. with effervescence. On recrystallization from acetone it melted at 219.5-221°C. Vargha gives the melting point at 220-222°C. The acetone derivative was converted to the free vitamin by dissolving in water and warming for 10 minutes in an evacuated flask. It was dried in a vacuum desiccator over barium oxide. Without further purification this slightly yellow product melted at 189.5°. The melting point of the pure substance is 192°C. The acidity and the amount of iodine reduced by these preparations were within 3% of the theoretical.

6890

Purgative Effect of Some Aliphatic Alcohols.

DAVID I. MACHT.

From the Pharmacological Research Laboratory, Hynson, Westcott and Dunning, Inc., Baltimore, Md.

In connection with a pharmacological study of the first 18 primary alcohols of the aliphatic series made in this laboratory in collaboration with Professor E. Emmet Reid of the Laboratory for Organic Chemistry, Johns Hopkins University, an investigation was also made of the higher members of the series, most of which are solids, to ascertain whether any of these exert a laxative or purgative action. The reason for such an inquiry was the finding by Macht and Barba-Gose, while studying the pharmacology of the oil of the *Ruvettus pretiosus* fish, that the active principle responsible for its laxative effect was the acetate of a hexadecyl or cetyl alcohol.[1] Because of the small amount of material available and the insolubility of these higher alcohols in water and physiological saline, the writers employed a new method.[2] Briefly, it consists of the introduction through a "stomach tube" of an emulsion of finely divided animal charcoal into the stomachs of full-grown white rats, previously fed on a standard dry diet composed of wheat (25 gm.), ground maize (25 gm.), rolled oats (28½ gm.), flaxseed meal (10 gm.) casein or whole milk, dried (10 gm.), sodium chloride (1 gm.), and calcium carbonate (0.5 gm.). An hour after the introduction of such an emulsion, the animal is quickly killed and the entire gastrointestinal tract is excised and stretched out on the operating table. The distance traversed by the black emulsion in a given time, easily ascertained by examination of the intestines, is measured in centimeters. Series of such experiments with a normal emulsion give consistent readings which are expressed in percentages of the total length of the intestinal tract. Other emulsions, similarly prepared, but in which 1% of the respective alcohols had been incorporated, are given other rats in the same way; and the distance traversed by the emulsion in one hour is also ascertained in each instance. In this way emulsions of all the alcohols studied were prepared and their passage through the intestinal canal of rats was compared with that of controls and also with that of emulsions containing olive oil, mineral oil and other well-known laxative drugs. The results obtained with the aliphatic alcohols are expressed in Table I.

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[1] Macht and Barba-Gose, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 772.

[2] Macht and Barba-Gose, *J. Am. Pharm. Assn.*, 1931, **20**, 558.

TABLE I.

Charcoal Emulsion	No. Experiments	Aver. Distance Traversed Hour % of total length of intestine
Normal	30	55
American mineral oil	5	56
Russian " "	5	56
Tetradecyl	10	65
Pentadecyl	10	64
Hexadecyl	15	69
Heptadecyl	10	63
Octodecyl	10	64

All the primary alcohols containing from 13 to 18 carbon atoms exhibited more or less laxative action. Hexadecyl or cetyl alcohol was the most effective in this respect. The action of the alcohol itself, however, was not as powerful as that of the ester, cetyl acetate, studied in the research on *Ruvettus* oil. This investigation brings to light a new property of some of the higher alcohols. It is well known that the old drug manna, which is a laxative, contains mannitol, a polyhydric alcohol, but it has not been known hitherto that the simple monohydric alcohols of the fatty acid series also produce a laxative effect. It is interesting to observe, furthermore, that when ordinary mineral oils were studied on rats by the method described above, no special laxative action was noted, thus confirming the generally accepted view that such mineral oils relieve constipation in human beings by virtue of their lubricating effect.

6891

Effect of Sensitization with Tuberculin Protein upon Development and Course of Experimental Tuberculosis.

FLORENCE B. SEIBERT.*

From the Henry Phipps Institute of the University of Pennsylvania.

The relation of tuberculin hypersensitiveness and resistance to tuberculosis has long been in dispute. Austrian[1] concluded that the state of hypersensitiveness to tuberculin protein afforded no protection against subsequent tuberculous infection and was even harmful. Krause and Willis[2] observed some lowered resistance to infection in animals sensitized to tuberculo-protein and more definitely in animals inoculated while suffering with anaphylactic shock. Their animals showed no cutaneous sensitivity. Nevertheless, they[3] attributed the usual state of resistance to reinfection in tuberculous animals directly to the effect of the allergic inflammatory reaction. Rich and McCordock[4] opposed this view on the basis of observations on experimental animals and human beings. Sabin, Smithburn and Geiger[5] noted no beneficial effect of the hypersensitive state on the course of experimental tuberculosis. Myers[6] and Stewart[7] have recently emphasized the predisposing effect of allergy for the development of adult type of tuberculosis. It has been shown[8] that upon repeated injections of sufficient quantity of highly antigenic preparations of tuberculin protein guinea pigs and rabbits will react cutaneously not only to this protein fraction, but also to the active material isolated from Old Tuberculin. It seemed desirable, therefore, to study the effect of this high degree of hypersensitiveness upon the tuberculous process.

Seventeen guinea pigs were sensitized with a tuberculin protein fraction, TPT,[8] by weekly intracutaneous injections of 10 mg. Six

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* Aided by a grant from the National Tuberculosis Association.

[1] Austrian, C. R., *Bull. Johns Hopkins Hosp.*, 1913, **24**, 11.

[2] Krause, A. K., *J. Med. Res.*, 1911, **24**, 361; 1916, **35**, 25. Krause, A. K., and Willis, H. S., *Am. Rev. Tuberc.*, 1919, **3**, 153.

[3] Krause, A. K., *Am. Rev. Tuberc.*, 1925, **11**, 343. Krause, A. K., and Willis, H. S., *Ibid.*, 1925, **11**, 439.

[4] Rich, A., and McCordock, H. A., *Bull. Johns Hopkins Hosp.*, 1929, **44**, 273.

[5] Sabin, F. R., Smithburn, K. C., and Geiger, J. T., *Trans. Nat. Tuberc. Assoc.*, 1933.

[6] Meyers, J. A., *Am. Rev. Tuberc.*, 1933, **27**, 121.

[7] Stewart, C. A., *J. Am. Med. Assn.*, 1933, **100**, 1077.

[8] Seibert, F. B., *J. Inf. Dis.*, 1932, **51**, 383.

TABLE I.

Course of Tuberculosis in Tuberculin Protein Sensitized and Non-sensitized Guinea Pigs.

Route of inoculation	Sensitized						Non-sensitized					
	Time of death after inoculation	days	Wt. of Spleen gms.	Extent of lesion in Spleen	Extent of lesion in Liver	Extent of lesion in Lungs	Time of death after inoculation	days	Wt. of Spleen gms.	Extent of lesion in Spleen	Extent of lesion in Liver	Extent of lesion in Lungs
Subcutaneously	Died	93	Large	+++	+++	+++	Killed	93	Med.	++	+	+
	"	94	12.6	+++	+++	++	"	94	2.4	+++	+	+
	"	101	10.0	+++	+	+	"	101	2.0	++	+	+
	"	125	21.6	+++	+++	+++	"	125	3.2	+	+	++
	Killed	185	5.9	+++	±	+++	Died	146	6.0	+	++	+
							Killed	185	4.5	++	+	+++
							"	193	6.6	+++	+	+
							"	193	9.5	+++	+++	+++
Intracutaneously	Died	91	5.2	+++	+++	+++	"	91	2.2	+++	+	+
	"	151	4.1	++	++	+++	Died	139	1.6	+	+	+++
	"	152	7.6	+++	+	+++	Killed	151	4.9	+++	+	+++
	"	159	13.9	+++	+++	+++	"	159	2.4	++	+	++
	Killed	193	5.3	+++	+	++	"	193	4.9	++	+	+++
	"	193	4.4	++	+	+++	"	193	4.1	+++	++	+++
							"	193	3.4	+++	++	—

of these died from intercurrent infection before significant tuberculous changes developed, and are omitted from the accompanying table. The first injection elicited very little, if any, cutaneous reaction; the next few injections were followed first by small and finally by large pale edematous reactions. After the seventh to the tenth injection, most of the animals gave reactions that were red and localized, intensely indurated and necrotic in the center, thus resembling very closely the severe reactions obtained with tuberculin in tuberculous animals. At no time did the animals display evidence of anaphylactic intoxication following the injections and, on the whole, they constantly gained weight. One week following the twelfth injection, 17 untreated and the 17 hypersensitive guinea pigs were inoculated with 0.001 mg. of a live virulent human-type tubercle bacillus, strain H 37, half of them subcutaneously and the other half intracutaneously. Following this whenever a sensitized animal died a control animal of approximately the same weight was killed, and finally, 6 months from the time of inoculation all surviving animals were killed.

In general those animals that had been sensitized and then inoculated subcutaneously, died sooner than the others. The most conspicuous change was great involvement of the spleen, as can be seen from the weights of this organ recorded in the table. The extensive distension of the splenic tissue by edema and hemorrhage and the diffuse caseation and necrosis with almost complete disappearance of normal tissue in the sensitized animals was in marked contrast to the predominating, discreet, raised and fibroplastic lesions found in the control spleens. Next in order of splenic involvement and in time of death were the sensitized guinea pigs that had been inoculated intracutaneously. After 6 months the lesions in the few surviving sensitized and in the non-sensitized control animals more nearly resembled each other and were in both groups of the chronic type.

These results, therefore, lead to the conclusion that a high degree of hypersensitiveness to tuberculin protein confers no immunity or increased resistance to subsequent tuberculous infection. On the contrary, it seems to hasten and extend the lesion and to be associated with much more extensive necrosis and caseation than is found in unsensitized animals. The experiment is being repeated and greatly extended.

6892

Monocytosis and Lymphopenia in Experimental Rabbit-Pox.

PAUL D. ROSAHN, C. K. HU AND LOUISE PEARCE.

From the Laboratories of the Rockefeller Institute for Medical Research.

During the course of an investigation of a spontaneous epidemic of rabbit-pox,[1, 2] observations were made on the blood picture of the experimental disease. Standardized pipettes and the supravital technique were employed in making the blood examinations. Striking quantitative changes were noted in two classes of cells. During the height of the disease there was a marked decrease in the number of lymphocytes and an enormous increase in the number of circulating monocytes.

In a group of 12 animals systematically studied, the lymphocytes fell from a mean preinoculation level of 2473 or 35.1% of the total count to 847 or 7.5% on the third day following intratesticular inoculation. This was an actual decrease of 65.8% or a relative decrease of 78.6% from the preinoculation lymphocyte level. Simultaneously monocytes rose from a preinoculation value of 374 (5.4%) to 1534 (12.4%) on the third day following inoculation, an actual increase of 310%, or a relative increase of 185% from the mean preinoculation finding. The total white count rose from a mean preinoculation level of 6,971 to 12,530 on the third day. The extreme potency of the virus is evidenced by the fact that 5 of the 12 animals died on the third day after inoculation, and all were dead on the fifth day.

Single observations were made on a large number of animals presenting a milder, more chronic form of the disease. Similar changes were observed. Lymphocyte values of less than 1000 per cm. and monocyte counts of more than 3000 were not uncommon. In relative per cent, the lymphocytes frequently composed less than 8% of the total count while monocytes sometimes rose to 40 and 50%.

Attention has been called to the monocyte-lymphocyte ratio in tuberculosis[3] and syphilis.[4] In the group of 12 systematically studied animals, the M/L index rose from a preinoculation value of

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[1] Greene, H. S. N., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 892.

[2] Pearce, L., Rosahn, P. D., and Hu, C. K., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 894.

[3] Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.

[4] Rosahn, P. D., and Pearce, L., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 869.

0.16 to 1.81 on the 3rd day after inoculation, while the highest M/L index in any animal of this group was 5.55.

An increase in monocytes has been reported in many diseases—tuberculosis, syphilis, virus III disease of rabbits, and small-pox. The suggestion has already been made that the proliferative phase of the general reaction is the feature common to these different diseases which may be associated with or responsible for the increased numbers of circulating monocytes. A decrease in the number of lymphocytes has also been reported in many diseases, but so far as we know in no disease has the decrease been so marked nor has it occurred in so short an interval as three days or less after inoculation.

6893

A Rapid Combined Slide Precipitation and Complement Fixation Test for Syphilis.

L. ROSENTHAL.

From the Laboratories of the Israel Zion Hospital, Brooklyn, N. Y.

The reaction between syphilitic serum and antigen manifests itself either in the formation of a precipitate or in fixation of an added complement. The fixation process is a prolonged one, requiring one hour or more, while the precipitation occurs almost immediately. Since in the syphilitic sera the same principle is accepted to be responsible for both the fixation of complement and flocculation, the reason for such a difference in the course of both reactions may depend on the concentration of the ingredients. In the various modifications of the Wassermann test the syphilitic serum, antigen and complement are used in diluted form, while the newest precipitation tests (Kahn, Citochol, Kline, Rosenthal) employ undiluted serum and concentrated antigen. It occurred to us that it might be possible to obtain a rapid fixation of complement by using the ingredients in the same concentration as in the precipitation tests. At the same time such a procedure would permit the combination of flocculation and complement fixation in one test.

Keining,[1] Kafka,[2] Kahn, Landau and McDermott,[3] in an attempt to combine complement fixation and flocculation added complement to a mixture of serum and antigen after the formation of a precipitate had already taken place. They then allowed an incubation of ½ to 1 hour for the fixation of the complement as in the classical Wassermann test.

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[1] Keining, E., *Deut. med. Woch.*, 1921, **47**, 157.

[2] Kafka, V., *Deut. med. Woch.*, 1921, **47**, 269.

[3] Kahn, R. L., Landau, T. L., McDermott, E., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 775.

In our preliminary experiments we learned that the mixture of syphilitic serum and antigen was frequently unable to fix the complement if the latter was added at the end of precipitation. We, therefore, concluded that the syphilitic serum must first be mixed with the complement and only then receive the antigen. In many controls we found that the presence of this complement in a mixture of syphilitic serum and antigen, even in amounts exceeding those used in our test, in no way interfered with the precipitation. In adopting the use of undiluted serum for complement fixation it was necessary to take into account that some human sera, when undiluted, have marked anticomplementary properties and inhibit the action of complement even in the absence of an antigen. This source of error can be eliminated, however. It is well known that the fixation of complement by an antigen-antibody complex is irreversible. The fixed complement cannot be liberated by subsequent dilution with saline. On the other hand, a complement inhibited by an anticomplementary serum regains its activity after dilution with saline (Bordet and Gay[4]). Therefore, in our test we add saline to the mixture of serum, complement and antigen after the precipitation takes place. As an additional safeguard to counteract the inhibitory properties of undiluted serum every specimen which is anticomplementary with one unit is tested subsequently with 2 units of complement. In the Wassermann tests the amount of complement used varies depending on its strength. In our test we found it more advisable to use a constant amount of complement in order to obtain a uniform dilution of every specimen of serum which is going to be examined. The adjustment of the hemolytic system is made by varying the concentration of red cells. Thus, for a very active complement a 5% suspension of red cells is used, and for a weak one a 2% or 1% suspension. A preliminary titration determines the optimal concentration of red cells.

The Test. 1. *Ingredients.* (a) The sera to be examined are inactivated at 56° for ½ hour. (b) Antigen: The antigen, as previously described,[5] consists of a mixture of equal parts of an alcoholic ether insoluble beefheart extract and a 2% solution of cholesterol in acetone. The antigen is diluted* with saline in the following manner: 1 cc. of antigen is pipetted into one test tube (4x5/8″) and 1.8 cc. of physiological salt solution into another. The contents of these 2 tubes are mixed by pouring from one into the other 8 times. The resulting emulsion is allowed to stay at room temperature for 10 min. to ripen and must be used within 30 min. (c) Hemolytic system: Guinea pig complement, antisheep hemolytic amboceptor (dilution containing 3 units per 1 cc.) and sheep red blood cells, as in usual Wassermann test.

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[4] Bordet, J., and Gay, F. P., *Annales Pasteur*, 1908, **22**, 625.

[5] Rosenthal, L., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 61.

II. *Glassware.* Glass slides (3¼x4″) mounted with 6 thin metal rings (1″ inside diameter). The rings are dipped in hot paraffin and placed on the slide. For measuring small quantities of complement and antigen we use graduated pipettes, whose pointed end is secured by hot paraffin in the hub of a stainless steel needle (Vim, gauge 25). Each drop of serum from this needle corresponds to 0.01 cc. and each drop of antigen to 0.005 cc.

III. *Procedure.* 1. Use 2 rings for each specimen. 2. Pipette 0.2 cc. of serum into each ring. 3. Add one drop of complement into each ring. 4. Add 2 drops of antigen emulsion into the first ring. Leave the second ring without antigen for control. 5. Rotate slide on a flat surface for 5 min. 6. Examine under the microscope (magnification 80) for formation of clumps in positive sera. 7. Dilute contents of every ring with 0.2 cc. of saline. 8. Pipette into every ring 0.1 cc. of sensitized red cells. 9. Slightly shake the slide and place into incubator at 37° for 15 min. 10. Examine under the microscope (magnification 80). In positive sera the red cells are unchanged and often clumped together, while in negative sera, as well as in controls, no red cells are to be seen as a result of complete hemolysis. With some experience it is easy to judge by the amount of unchanged red cells the degree of inhibition of hemolysis.

The test was performed on 165 syphilitic sera and 220 normal sera, and gave results corresponding with those obtained independently by the Wassermann and Rosenthal precipitation test.

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* Originally for the precipitation test for syphilis we used the antigen in undiluted form. Subsequently in collaboration with Dr. Rein we adopted the dilution of antigen with saline.

6894

Preparation of Vitamin C from Lemon Juice.

W. A. WAUGH,* O. A. BESSEY* AND C. G. KING.

From the Department of Chemistry, University of Pittsburgh.

The isolation and identification of vitamin C[1] has stimulated research in many laboratories where the crystalline substance is desired. Our original procedure has been successfully followed in other laboratories, but the present detailed procedure can be followed more readily, with larger yields, and in less time. Working with one to 6 liters of lemon juice, consistent yields of 100 to 150 mg. per liter of the crystalline vitamin may be regularly obtained.

Filter 3 l. of juice through muslin, add 7 gm. of copper-free basic lead carbonate ($2PbCO_3.Pb(OH)_2$) per 100 cc., stir with an electric stirrer for 3 hr., add saturated neutral lead acetate solution (about 500 cc. per 3 l. preparation), continue stirring for 30 min., and centrifuge (ppt. is inactive, but may be rinsed for further recovery). Maintain a stream of CO_2 bubbling through the liquid while cooling to 0°C., add NH_4OH (1:3), with constant stirring, to the liquid until the pH reaches 7.6 (as shown by phenol red on a spot plate), centrifuge, discard the liquid (inactive), redissolve the yellow precipitate with acetic acid (1:3), and reprecipitate with NH_4OH at a pH of 7.6. (If either of the above liquid portions remain yellow, probably not enough lead acetate solution or ammonia was added). Dissolve the precipitate slowly with hydrochloric acid (1:1), adding acid until the solution will turn Congo red distinctly blue. Extract the entire preparation with one-half volume of n-butyl alcohol (to remove oils, etc.) and then add ethyl alcohol until the concentration is about 75%. Centrifuge (ppt. is mostly $PbCl_2$), evaporate to about 10 cc., add 100 cc. of acetone, centrifuge (ppt. mostly inorganic salts), add enough $BaCO_3$ to neutralize free HCl (to Congo red), and evaporate to dryness in the presence of fine white sand (about 5 gm.). Add 15 cc. of dry benzene and again evaporate to dryness. Cover and permit to extract over night with

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* The authors are greatly indebted to Parke, Davis & Company, and The Abbott Laboratories, for a Research Fellowship Grant during the course of this investigation, and to the California Fruit Growers Exchange for supplying the lemons used.

[1] *Science*, 1932, **75**, 357; 630.; *J. Biol. Chem.*, 1932, **97**, 325; *Cf., Ann. Repts. Soc. Chem. Ind.*, 1932, 547.

200 cc. of absolute acetone (the organic residue is largely inosite†). Rinse, centrifuge, and evaporate the solution to dryness. Dissolve the residue in 30 cc. of absolute n-propyl alcohol, cool to 0°C. and add an equal volume of petroleum ether (B.P. 30-60°). Centrifuge off the precipitate and add to the solution 60 cc. more of the petroleum ether, keeping the solution near 0° (most of the vitamin remains in solution). Extract the 2 separate precipitates in turn with 25 cc. of n-propyl alcohol, for about 10 min., at room temp., cool, and again precipitate slowly by the addition of 75 cc. of petroleum ether. Centrifuge, rinse, and discard the precipitate (after testing for reducing action). Combine the 3 liquid portions and evaporate to dryness. Add 15 cc. of dry benzene and again evaporate completely. Redissolve in a minimum volume of n-propyl alcohol, cool, and add petroleum ether (B.P. 135-150°) until it begins to cloud slightly, and let stand in an open beaker in a desiccator over P_2O_5 in a refrigerator. If crystallization is not practically complete within 24 hr. evaporate the alcohol slowly in a vacuum desiccator. For recrystallization methyl alcohol may be used satisfactorily. The crystals may be rinsed with ice-cold glacial acetic acid or acetone.

Notes. The water used for preparing solutions should be redistilled from glass, and all reagents, especially the lead salts should be free from copper. The acetone, propyl alcohol, and petroleum ether should be carefully dried, or crystallization will not be satisfactory. An excess of HCl during acetone and subsequent evaporations will cause darkening and the formation of resinous material. If a second liquid phase appears during the first acetone separation, the aqueous phase should be further extracted or there will be a serious loss of the vitamin in the syrupy layer which occasionally forms at the bottom of the solution. Evaporations should be carried out under reduced pressure (below about 40°), with a capillary tube carrying a stream of CO_2. It is possible to check each step in the procedure by titrating small aliquot portions with acid iodine solution or with 2, 6-dichloro-phenol-indophenol,[2] in case any question arises concerning a loss in activity. In our experience titration with the dye has been satisfactory as an index of the vitamin C content of both plant and animal tissues, but the iodine titration is only satisfactory with purified material. Centrifuging precipitates instead of filtering facilitates protection from air and moisture, and working rapidly at lower temperatures.

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† Identified by Dr. E. K. Nelson (private communication).

[2] Gibbs, Cohen and Cannon, *Public Health Reports*, 1925, **40**, 649; Tillmans, Hirsch and Hirsch, *Z. Untersuch. Lebensm.*, 1932, **63**, 1; Mottern, Nelson and Walker, *J. Assn. Off. Agr. Chem.*, 1932, 614; Birch and Dann, *Nature*, 1933, **131**, 469.

6895

Amino Acids as Gastric Secretagogues.

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From the Department of Physiological Chemistry, Yale University.

In the course of our recent studies on the secretion of gastric pepsin, we examined the effects of small amounts of amino-acids administered by the technique of Tagawa[1] to Pavlov gastric pouch dogs. The volumes of juice obtained were too small to allow accurate calculations of the total pepsin contents to be made, and the volume of secretion obtained differed greatly from that reported by Tagawa. In the present study 13 amino-acids were tested: alanine, arginine monohydrochloride, aspartic acid, cystine, glutamic acid, glutamic acid hydrochloride, glycine, histidine monohydrochloride, leucine, isoleucine, phenylalanine, tyrosine and valine.

As has been emphasized by Ivy and Javois,[2] all of the amino-acids appeared to be rather weak secretagogues with but few exceptions. Contrary to the results of Tagawa, glutamic acid hydrochloride was found in our work to be the weakest instead of the strongest stimulant for gastric secretion. All of the free acids, with the exception of isoleucine, were found to be slightly more active than glutamic acid hydrochloride. The fact that glutamic acid was a more potent stimulus for secretion than its hydrochloride is directly opposite to the findings of Tagawa.

Isoleucine, arginine and histidine were found to produce from 2 to 3 times the volume of juice excited by the other amino-acids tested. The activity of histidine in this respect is particularly interesting in view of its close structural relationship to histamine. The potency of isoleucine argues against the view that the basicity of the other 2 active amino-acids is the chief factor upon which their secretagoguic activity depends.

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* These data form a part of the dissertation submitted by Elizabeth R. B. Smith to the Faculty of the Graduate School, Yale University, May, 1933, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[1] Tagawa, J., *Biochem. Z.*, 1931, **243**, 344.

[2] Ivy, A. C., and Javois, A. J., *Am. J. Physiol.*, 1924, **72**, 591.

These studies were conducted on 8 Pavlov gastric pouch dogs, at least 3 being used for each amino-acid studied, and 2 of the animals serving throughout the entire series of observations. Secretion was followed for 6 hours subsequent to the administration of 100 cc. portions of M/20 amino-acid solutions by stomach tube to the fasting animals. The test solutions were always given after a preliminary observation period had revealed that the gastric glands were in a resting condition.

6896

Meat Extractives as Stimulants for Secretion of Pepsin.

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From the Department of Physiological Chemistry, Yale University.

As a result of the experiments conducted by Lobassow,[1] Sirotinin,[1] and of other workers in Pavlov's laboratory, and of the fractionation studies of Krimberg and Komarov[2] and others, the extractives of muscle have been recognized as potent stimuli for the secretion of gastric juice; their relation to the secretion of the particular component of gastric juice, pepsin, has received much less attention. We have conducted experiments on Pavlov gastric pouch dogs, using solutions of 4 different muscle extractive preparations and of 3 of these combined with starch, to determine what effect these materials might have on the secretion of pepsin. Secretion was followed during a preliminary period and then for 6 hours after administration of the test solution, and, in some cases, a second dose of the test material was given. At least 3 animals were used in the tests of each preparation and 2 were subjects throughout the entire study. Free and total acidities were titrated to the endpoints of Töpfer's reagent and of phenolphthalein respectively; total chloride concentration was determined by the method of Van Slyke[3] and peptic activity by the procedure of Gilman and Cowgill.[4]

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* These data form a part of the dissertation submitted by Elizabeth R. B. Smith to the Faculty of the Graduate School, Yale University, in partial fulfillment of the requirements for the degree of doctor of philosophy, May, 1933.

[1] Babkin, B. P., *Die Äussere Sekretion der Verdauungsdrüsen*, Berlin, 1928.

[2] Krimberg, R., and Komarov, S. A.. *Biochem. Z.*, 1926, **176**, 73; *Ibid.*, 1928, **194**. 410.

The first commercial meat extract† to be examined stimulated the flow of juice as determined by volume, but not the secretion of pepsin unless starch was added to the solution (Table I). In the latter case, we found, as had Lobassow,[1] that the enzyme content of the juice was markedly increased suggesting the stimulation of the flow of pepsin through mechanical factors.

TABLE I.
Secretion of Gastric Juice and of Pepsin during the First Hour after Administration of Various Preparations of Muscle Extractives.

	Gastric Juice			
Preparation Used	Volume cc./hr.	Acid m.-eq.*	Chloride m.-eq.	Total Pepsin units
Meat extract: "Lemco"	7.5	101	159	750
"Lemco" with starch	8.1	113	152	1013
Meat extract: "Armour"	7.3	111	157	730
"Armour" with starch	7.5	100	159	600
Meat stock: "Homemade"	7.5	112	160	750
Meat extract, fluid: "Valentine's"	7.4	115	161	864
"Valentine's" with starch	7.7	106	159	880

* Milli-equivalents per liter. See Kalkstein, D., *Yale J. Biol. and Med.*, 1930, **2**, 353.

Trials with another commercial meat extract‡ likewise gave negative results as regards the secretion of pepsin. With this material the peptogoguic activity was absent, whether the test material was given alone or was administered together with starch, although the flow of gastric juice was considerable during the first hour after administration (Table I).

A meat stock preparation made in the laboratory from defatted fresh beef muscle was no more effective in promoting the secretion of pepsin than were the 2 commercial preparations mentioned above (Table I).

The fourth material investigated was a commercial preparation of the extractives marketed in fluid form.§ In contrast to the others tested, this product appeared to promote the secretion of gastric pepsin. A second administration of the test solution at the end

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[3] Van Slyke, D. D., *J. Biol. Chem.*, 1923, **58**, 523.

[4] Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 742.

† "Lemco" made in Argentina from beef muscle and repacked in London, England, by Oxo, Limited.

‡ Furnished by Armour and Company, Chicago, Ill.

§ Furnished by Mr. Braxton Valentine of the Valentine Meat Juice Company, Richmond, Virginia.

TABLE II.
A typical protocol showing effect of administration of fluid preparation of muscle extractives (Valentine's) on secretion of gastric juice, and particularly its constituent pepsin.

		Gastric Juice					
		Chloride	Acid		Pepsin		Remarks
Time hr.	Volume cc./hr.	total m.-eq.*	Free m.-eq.	Total m.-eq.	Per cc. units	Total units	
							15 cc. "meat juice" with 35 cc. water by stomach tube
1	5.2	159	108	118	150	780	No mucus
2	1.5	155	77	91	100	150	" "
3	0.3	—	—	—	100	—	" "
							Dose repeated
4	4.0	161	97	112	200	800	No mucus
5	0.2	—	—	—	100	—	" "
6	0.4	—	—	—	92	—	" "

*Milli-equivalents. See footnote to table I.

of the third hour of the experimental period resulted in an increase in the pepsin concentration as well as in the total amount of pepsin secreted, so that the values for the fourth hour equalled or exceeded those of the first hour (Table II). Inasmuch as this second dose was effective, the apparent peptic increase could not be due simply to a flushing effect on the glands. If, however, the second dose was given at the end of the second instead of the third hour of the secretory period, no increase in either the flow of juice or the secretion of pepsin could be observed. There may be, therefore, a refractory period for these glands during which they do not react to a repeated stimulus. Administration of starch along with the fluid preparation of extractives did not markedly increase the amount of pepsin secreted (Table I), indicating that in this case the stimulation by the chemical exciter, *i. e.*, the meat extract, has approached the physiological maximum so that the addition of a further mechanical stimulation does not increase the enzyme output. It would seem from these results that the findings of Zeliony and Sawitsch[5] and others, that mechanical stimulation is the principal factor in the secretion of gastric pepsin, do not account fully for all the factors involved, because in our study a definite chemical excitation has been induced.

The quantities of the 4 extractive preparations given to the dogs were such that the secretion during the first hour after administration was approximately the same for each in a given animal (Table I). Therefore, the positive peptogoguic effect observed with the fluid preparation of extractives could not have been due to a more massive dose in this instance.

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[5] Zeliony, G. P., and Sawitsch, W. W., *Compt. rend. soc. biol.*, 1914, **77**, 50.

These experiments are purely preliminary in nature. Further studies are necessary in order to determine the nature of the peptogoguic substance. Fractionation of the fluid extract and careful study of the action of each fraction will be required.

6897

On the Digestion of Animal Forms by the Oyster.

T. C. NELSON.

From the Department of Zoology, Rutgers University.

Numerous workers have interpreted living organisms in the faeces of oysters as proof of unavailability of such forms as food. Nelson[1] demonstrates that living organisms in the faeces of the oyster are the result of the incomplete separation of food particles from undigestible matter, inherent in the oyster's feeding mechanism. Protozoa, rotifers, nematodes, copepods and their larvae, larvae of polychaete worms, of snails, clams. oysters and tunicates and even small fish eggs comprise up to 80% of the stomach contents in summer. All of these organisms save the lamellibranch larvae were disintegrating, hence it was concluded that they were being digested. Savage[2] found only 10% organisms in the stomach contents of oysters in English waters. Yonge[3] reports extensive studies on the enzymes of the digestive tract of the oyster and of related molluscs. The extracellular enzymes are a powerful amylase and a glycogenase, both derived from the crystalline style. Berkeley's[4] finding of an oxidase in the style is confirmed, but no extracellular lipase or proteose could be demonstrated. Yonge confirms for the oyster the work of List[5] on the phagocytic activity of the "digestive gland" of Mytilus and he extends the observations of Vonk[3] on the importance of phagocytes in digestion in the oyster.

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[1] Nelson, T. C., *J. Morph.*, 1918, **31**, 53; *Rpt. N. J. Agr. Expt. Sta.*, 1920, **325**; PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 166; *Biol. Bull.*, 1925, **59**, 86.

[2] Savage, *Ministry Agr. and Fish*, 1925, **8**, Ser. 2. No. 1. London.

[3] Yonge, C. M., *J. du Cons. Internat.*, 1931, **6**, 175 (includes all other references).

[4] Berkeley, C., *J. Exp. Zool.*, 1923, **38**, 477.

[5] List, T., *Fauna u. Flora d. Golfes v. Neapel*, 1902, **27**.

Yonge concludes[3] that lamellibranchs are "specialized herbivores capable of the extracellular digestion only of starch or glycogen," and he accounts for the presence of living organisms in the faeces as due to "complete absence of extracellular protease and lipase".

Our own experiments on both the European oyster, *Ostrea edulis*, and on the American oyster, *O. virginica*, show that Yonge has not fully appreciated the power and the importance of the style enzymes in extracellular digestion in the oyster; and that his classing the oyster as wholly herbivorous is not in accord with the facts. Large diatoms, such as *Coscinodiscus*, and long filamentous diatoms, such as *Skeletonema* and *Melosira* are digested extracellularly by style enzymes. When *Skeletonema* dominates the plankton, the stomach contents of oysters are a veritable soup of this form in all stages of digestion. Both species of oyster, under conditions at Delaware Bay, N. J., show during summer months many disintegrating animal forms in the stomach. One outstanding case was an *O. edulis* which on August 22nd yielded 0.75 ml. of rich brown stomach contents, mostly the free-living nematode, *Chromadora* sp. Analysis showed 160 worms alive and active, 471 dead but intact, and 5,842 partially or wholly disintegrated. This oyster was feeding actively when taken, and the stomach contents were under observation less than 2 minutes later. The nematodes were swarming in the algal growths on the oyster's shell. Since oysters do not feed during the latter part of the night, this oyster had gathered these worms

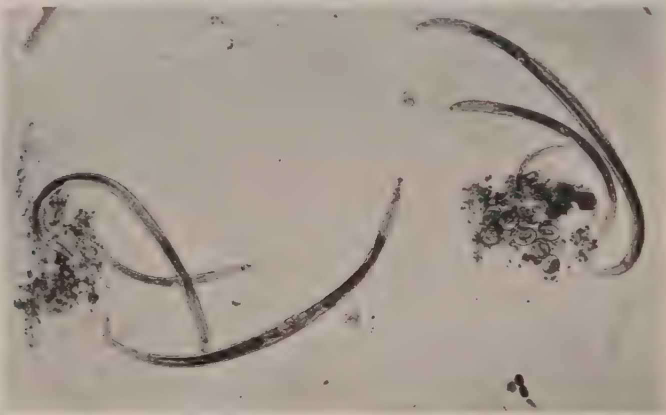

FIG. 1.
Chromadora sp., and embryonated eggs of the same nematode from decaying *Ulva*, ×96.

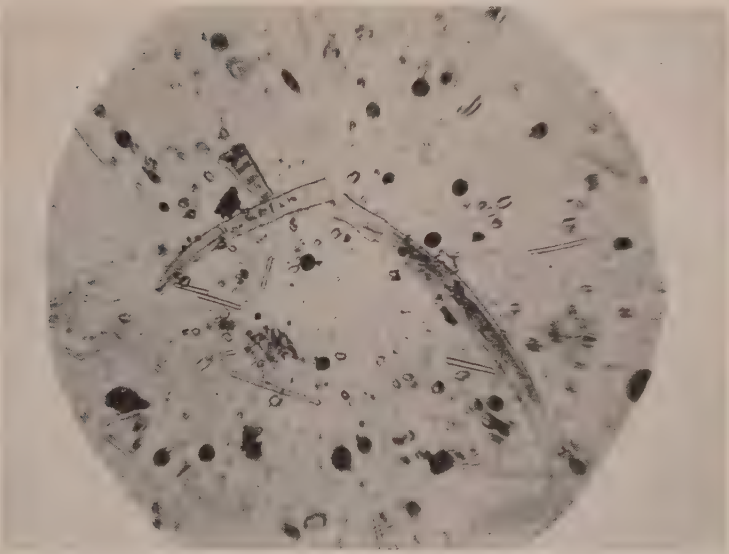

FIG. 2.

One of more than 5000 disintegrating *Chromadora* from the stomach of *Ostrea edulis*. A small portion of the anterior end of a second worm also is present, ×144.

between sunrise and 11:00 a. m., when the test was made. The stomach contents were kept at room temperature for 24 hours but no further disintegration of the worms occurred, indicating cessation of enzyme action. Death due to oxygen lack, followed by bacterial action cannot account for the disintegration of these nematodes, the process was much too rapid. In every instance disintegration was preceded by wrinkling and subsequent bursting of the integument of the worm, liberating the body contents as small granules. These were of such size as readily to admit of phagocytosis.

It is evident, therefore, that some substance or substances present in the stomach of an oyster with a well formed style can penetrate the chitin of crustacea and the cuticle of nematodes resulting in death and disintegration of the animals. This serves the same purpose as the crushing action of the gizzard-like stomach of carnivorous Septibranchs so admirably demonstrated by Yonge, since it reduces the prey to particles small enough to be engulfed by phagocytes. Animal forms both in the plankton and on the bottom at times become very numerous during the warm months when the oyster's ciliary currents are of such strength as to capture these active organisms. That such acquisition of protein is important to the oyster in the late summer after its exhaustion due to repeated spawning cannot be doubted.

6898

Age and Resistance to Ether in Mice.

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From the Biological Laboratory, Brown University.

Wide variations have been observed in the length of time necessary to anesthetize mice by means of ether. Active adults as well as old and rather feeble animals reacted in perhaps one-tenth of the time of young a week or less in age. Two groups of mice were available for testing this observation, and a series of experiments was made with each group.

The first of these groups (here designated the K Strain) was a genetically heterogeneous lot, containing various combinations of the following mutant types: chinchilla, extreme-dilution, albinism, white-bellied-agouti, non-agouti, brown, short-ear, piebald, silver, dwarf, flexed-tail, and several new types of which the genetics is not fully worked out. A number of these mice were from the stocks of Dr. G. D. Snell of the University of Texas, to whom the author is greatly indebted for assistance in many ways. The second group (here designated the Inbred Strain) was, on the other hand, relatively homogeneous genetically, as a result of some 25 generations of inbreeding, and contained only the wild agouti type. These mice were kindly sent to the author by Dr. L. C. Strong of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

The apparatus consisted of several 3-liter flasks with rubber stoppers; fresh "Ether Squibb"; a graduated pipette; platform scales; thermometer. The procedure was as follows: Into one of the flasks at room temperature (23°-26°C.) was introduced 2.4 cc. of liquid ether, which was allowed to vaporize while the stopper was loosely in place. Then with the stopper tight, the flask was inverted for one minute, after which it was allowed to stand for a further 10 to 15 minutes, when the temperature within the flask was about 2° lower than room temperature. With the flask inclined to permit the mouse to slide rather than drop to the bottom, the stopper was removed, the mouse introduced as quickly as possible, and the stopper immediately replaced. The time of introduction was recorded. When immobilized, the mouse (still within the flask) was rolled over in order to allow observation of the movements of the abdomen. The cessation of breathing (the assumed death point) was taken as the time at which these breathing movements of the abdomen were no longer detectable. This time was recorded to the nearest 5 seconds. The animal was then removed and weighed.

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The results with the K strain (87 individuals) showed a definite negative correlation between age and cessation of breathing under etherization. Mice under 8 days of age ceased breathing in general only after 30 to 60 minutes; at 15 to 17 days of age cessation occurred in 5 to 10 minutes; and from 21 days to 32 days in 3 to 5 minutes. Higher age groups showed much less uniformity but most animals in these groups gave records of 3 to 6 minutes.

Averages for the different age groups (up to 32 days) are plotted in Curve A, Figure 1. There are many irregularities, some of which were probably caused by such interrelated factors as differences in weight, early or late maturing, number of sibs, and variation in the endocrine balance.[1] Since these factors might have a genetic basis, it seemed highly desirable to repeat the experiment upon a long-inbred strain of mice.

The results of the second series from the Inbred Strain (52 individuals) are shown in Curve B, Figure 1. As compared with Series K two differences appeared in this inbred series,—there was much less variation, and cessation of breathing occurred considerably later in the lower age groups. Young of lighter weight have greater resistance, the heavier animals generally behaving as though they were slightly older than their lighter sibs.

Sumner[2] gives information which suggests an explanation for the age differences in the susceptibility of mice to ether, that in young mice the heat-regulating mechanism of the body is poorly developed. A graph of Sumner's recordings (Curve S, Figure 1) of the body temperatures of mice is herewith combined with the author's observations of the effect of age on etherization-time. The line of the Inbred Strain follows Sumner's line so closely as to be

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[1] Scott, W. J. M., *J. Exp. Med.*, 1923, **38**, 543.

[2] Sumner, F. G., *J. Zool.*, 1913, **15**, 348.

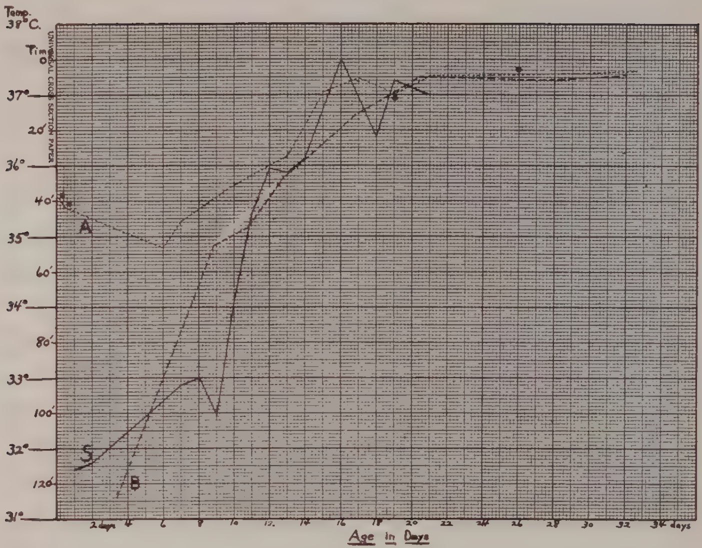

FIG. 1.

Age, Etherization-Time and Body Temperature of Mice.

The solid line "S" gives the body temperature of mice up to 3 weeks of age, as given by Sumner.[2]

Broken line "A" gives the time of cessation of breathing by etherization at various ages (up to 32½ days) of the K Strain.

Broken line "B" gives the cessation of breathing by etherization at various ages (up to 32 days) of the Inbred Strain, except that mice differing in age by three-tenths of a day or less, are charted as if belonging to the same age group.

* Indicates a point determined by only a single individual, and hence unreliable as a part of the curve.

strongly suggestive that the reason for the slower rate of etherization in the young mice is a lowered metabolism associated with a drop in their body temperatures after removal from the nest—the removal occurring about 5 minutes before the introduction into the flask.

The author hopes to obtain further evidence upon this explanation by running a similar Inbred Strain series at a temperature of 37°C.

The results of Avery and Johlin[3] in their tests with nitrogen, argon, hydrogen, carbon dioxide, carbon monoxide and illuminating gas, may possibly be similarly explained.

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[3] Avery, R. C., and Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1184.

6899

Conversion of Osteolytic Carcinoma Metastases to Bone into Osteoblastic Ones by Large Doses of Calcium.

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The administration of calcium in large doses is beneficial in many cases of advanced malignant neoplastic disease. Pain is partially or completely relieved and there is a temporary improvement in the patient's general condition. The use of calcium in such patients rests entirely on an empirical basis, since there is no satisfactory explanation for its temporary beneficial effects. The following case-history is reported because of the influence calcium apparently had on metastatic carcinoma in bone.

Z. P. White female age 44. The patient had a radical mastectomy in 1930 for carcinoma of the right breast. This was followed by 10 deep X-ray treatments to the upper right chest. There were no complaints until the spring of 1932 when severe aching pains developed in the pelvis, thighs and knees. After administration of sedatives this disappeared until the fall. She was seen at the University of Chicago Clinics October 7, 1932, complaining of severe pains as described above, of 3 weeks' duration, necessitating almost continuous rest in bed. Other complaints were sleeplessness due to pain, inappetence, loss of weight and inability to stand or walk.

Examination revealed a middle-aged woman suffering from pain referred to the pelvis, thighs and lower back. Roentgenograms of the pelvis showed extensive osteolytic and osteoblastic metastases in the pelvis and upper femurs. Especially prominent were osteolytic metastases in the pubic bones on each side of the symphysis, a large osteolytic metastasis in the anterior superior spine of the left iliac bone and a large osteoblastic metastasis in the trochanteric region of the left femur.

The patient was given 12 intravenous injections of 5 cc. ten per cent calcium gluconate 3 times a week, and at the same time began taking calcium lactate by mouth, 1 gm. after meals 3 times a day. The dose was increased until she is now taking 3 gm. after meals 3 times a day.

Three weeks later there was considerable improvement. This has continued until the present time. The patient was last seen

April 7, 1933. There was no pain. She walks normally, does her own domestic work and spends several hours a day working outside the home. Her appetite is very good and she has gained 27 pounds since last October. At this date, 6 months after calcium was instituted, the patient having received her regular dose the day before, and blood drawn before breakfast, the blood calcium was 10.5 mg. per 100 cc. Blood phosphorus 4.2 mg. per 100 cc.

Roentgenograms (April 7, 1933) of the pelvis and upper femurs reveal a diffuse sclerosis of the bones. Most of the osteolytic metastases appear now as osteoblastic. The solitary osteoblastic metastasis in the upper left femur has not increased in size. It is impossible to state definitely if the other metastases have changed in size. Roentgenograms of the lungs show no metastases present.

The writer has been unable to find record of an instance of osteolytic metastases spontaneously changing into osteoblastic ones, or vice versa. It is generally held that this does not occur. X-ray therapy may cause sclerosis of an osteolytic carcinoma metastasis, but this must be regarded more in the nature of a healing process than a change in the bone destroying or stimulating properties of the carcinoma cells.

There is no other factor that could have caused the changes in the above patient, except the calcium administered continuously in large doses. The results in this case warrant the administration of calcium in large doses to all patients exhibiting skeletal metastases from carcinoma.

6900

Local Anesthetic Properties of Some Aliphatic Alcohols

DAVID I. MACHT AND MARY E. DAVIS.

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Macht called attention to the fact that it is not necessary to have complex chemical compound containing nitrogen in its molecule to produce local anesthesia. He showed that the simple aromatic compound, benzyl alcohol, exhibited marked local anesthetic properties when studied by different pharmacological methods.[1] During a pharmacological study of 23 isomeric octyl alcohols, Macht and Schroeder discovered that these straight chain alcohols all produced marked local anesthesia and, furthermore, that combinations of these acted synergistically.[2] These findings suggested an inquiry into the possible local anesthetic properties of other aliphatic alcohols. In connection with a study of 18 primary aliphatic alcohols of the fatty acid series made jointly in the Laboratory for Organic Chemistry, directed by Professor E. Emmet Reid, Johns Hopkins University, and in this pharmacological laboratory, tests were made to determine whether any of these alcohols exerted a local anesthetic action.

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[1] Macht, *J. Pharmacol. and Exp. Therap.*, 1918, **11**, 263.

As a starting point for such experiments, 1% solutions of the 18 aliphatic alcohols were made in 95% ethanol. For the study of their local anesthetic effect on animals these were diluted 1 to 10, yielding a concentration of the alcohols of 1:1,000 in 9.5% ethanol. Alcohols containing more than 10 carbon atoms, which were not soluble to the extent of 1:1,000, were used in saturated solution. Pharmacological tests were first made on the conjunctival and corneal reactions of rabbits and then on living frogskin by well-known standard methods. While studying the anesthetic effect on frogskin, we made a note of (1) the rapidity of onset of anesthesia, (2) the duration of complete anesthesia, (3) the time of cessation of all anesthetic effect, and (4) the concentration of the alcohol used. The subjoined table shows the results obtained. Seven of the aliphatic alcohols studied exerted a local anesthetic action. Beginning with the most potent and ending with the least potent, they were as follows: octyl, heptyl, nonyl, decyl, undecyl, dodecyl and hexyl. Neither the propyl nor butyl nor amyl alcohols of the primary, secondary or tertiary varieties, nor alcohols containing from 13 to 18 carbon atoms exhibited any local anesthetic effect.

TABLE I.
Anesthesia for Frogskin.

Primary Alcohol	No. Experiments	Time of Exposure min.	Duration of Complete Anesthesia*	Cessation of All Anesthesia min.
Hexyl, 1: 500	10	5	Slight anesthesia	—
Heptyl, 1:1000	10	1	10 min.	24
Octyl, 1:1000	10	1	15 "	35
Nonyl, 1:1000	10	1	5 "	18
Decyl, 1:1000	10	2	2 "	5
Undecyl, 1:1000	5	5	Slight anesthesia	—

*Variations in readings not more than 10%.

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[2] Macht and Schroeder, *Arch. f. exp. Pathol. u. Pharmakol.*, 1930, **158**, 53.

6901

Denervation of Carotid Sinuses and Section of Depressor Nerves to Produce Chronic Arterial Hypertension.

A. DE GROAT, H. H. DAVIS AND C. H. MC DONALD. (Introduced by B. Sure.)

From the Departments of Physiology and Pathology, University of Arkansas School of Medicine.

From the investigations of Hering, Heymans[1] and their collaborators[2] on the reflexes originating in the carotid sinuses came a method that promised to make possible the study of the pathological changes which may result from chronic, arterial hypertension, uncomplicated by the action of infectious or toxic agents. By denervating the carotid sinuses and sectioning the depressor nerves (rabbit) or the vagi (dog) these authors obtained the equivalent of a permanently low intracarotid pressure, namely, permanent arterial hypertension, hyperpnea, and hyperepinephrinemia. The hypertension was apparently demonstrated to be the direct result of the increased secretion of epinephrine. Unless the denervation was performed in stages, the animal was said to succumb to acute cardiac dilatation. The respiratory effects of the denervation consisted of rapid, labored breathing with lowering of the alveolar CO_2.

The method was applied to the investigation of essential hypertension and arteriosclerosis with interesting and apparently positive results.

Experiments were planned to determine precisely the course of the blood pressure after denervation of the carotid sinuses and section of the depressor nerves, and to explore the possibilities in the operation for the production of lesions in the rabbit. Fourteen rabbits were successfully denervated either on one or both sides. Ten animals were operated upon in 2 stages, the second following the first by an interval of 5 to 10 days. Four animals were denervated in one stage. Blood pressure determinations by the McGregor[3] method were made repeatedly before and after the operations. After bilateral denervation in 9 rabbits, slight elevations of pressure were observed in 5. The degrees of elevation of the pressure and the time required for the pressure to return to normal were as follows:

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[1] Heymans, C., *Compt. rend. Soc. de biol.*, 1931, **107**, 1293.

[2] Goormaghtigh, N., *Ann. d'anat. path.*, 1931, **8**, 585.

[3] McGregor, L., *Arch. Path.*, 1928, **5**, 630.

36 mm., 6 days; 30 mm., 90 days; 12 mm., 28 days; 10 mm., 22 days; 14 mm., 3 days. Precautions were taken to avoid the errors of observation into which the McGregor method may easily lead when moderate changes in blood pressure are involved. Bilateral denervation, performed in one stage, was quite as well tolerated as unilateral denervation. No changes in the respiratory rates were observed after the rabbits had recovered from the immediate effects of the operation.

To detect any modification in the secretion of epinephrine, the splenic volume, as revealed by the erythrocyte count of the peripheral blood, was utilized. The blood was drawn approximately 15 minutes after completion of the denervation. After unilateral denervation, no changes were observed in the erythrocyte counts. Bilateral denervation in 9 animals was followed by a polycythemia in 5. The maximum increase in the erythrocytes was 1,180,000, the minimum, 420,000. Blood sugar determinations, made after complete recovery of the animals from the operation, were always normal (80 to 105.6).

6902

Effect of Vitamin Deficiency on Gastric Secretion.

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Voegtlin and Myers[1] suggested the identity of vitamin B and secretin, but this could not be confirmed by Anrep and Drummond,[2] Downs and Eddy[3] nor Cowgill and Mendel,[4] who reported that this vitamin exhibits no effect on the liver, salivary or pancreatic secretions. Cowgill, Deuel and Smith[5] state that the restorative action of vitamin B on the appetite is not due to stimulation of gastric secretion, but they made no investigation of the stomach secretions. Miyadere[6] found a great diminution of the secretion from the Pavlov pouch on a vitamin-free diet, yet 100 cc. of 5% alcohol introduced into the main stomach provoked a flow of gastric juice. Farnum[7] reported a diminution in the volume and acidity of the secretion from Pavlov pouches in dogs kept on a beri-beri diet in response to a standard autoclaved meal, to histamine, but not to "gastrin." Her results were somewhat irregular, however, and not quite conclusive. Recently Cowgill[8] quoted Gilman's thesis of 1931 reporting the development of gastric achlorhydria in animals fed on diets lacking vitamin B.

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[1] Voegtlin, C., and Myers, C. N., *J. Pharmacol.*, 1919, **13**, 301.

[2] Anrep, G. V., and Drummond, J. C., *J. Physiol.*, 1921, **54**, 349.

[3] Downs, A. W., and Eddy, N. B., *Am. J. Physiol.*, 1921, **58**, 296.

[4] Cowgill, G. R., and Mendel, L. B., *Am. J. Physiol.*, 1921, **58**, 131.

[5] Cowgill, G. R., Deuel, H. J., and Smith, A. H., *Am. J. Physiol.*, 1925, **73**, 106.

[6] Miyadere, K., *Biochem. Z.*, 1921, **124**, 244.

Three dogs with oesophagotomy, gastric fistula and obstructed pylorus were maintained by us in good health for several months with duodenal feedings. One animal had also the pyloric antrum resected. These animals offered an excellent opportunity for the study of vitamin-deficient diets, since ample nourishment could be introduced independently of the capriciousness of the dogs' appetite or its disappearance in vitamin B deficiency. Many abnormalities in animals deprived of vitamin B might be the result of inanition arising from anorexia and not the specific effect of vitamin B deficiency. With the intestinal feedings the amount of food introduced was maintained on the same level during the whole experiment which lasted in each case for more than a month. The loss of weight during this period was insignificant. In one dog the weight varied from 16.1 kilos to 15.8 kilos, in the second from 16 to 15 kilos, while the third dog is still under experiment. None of these animals lost their appetite but eagerly ate all meat offered them in the sham-feeding experiments.

In all 3 animals the results obtained were the same. The experiment consisted of removing all vitamins from the dog's diet. The gastric function was tested by sham-feeding (nervous phase), subcutaneous injections of histamine and the introduction of alcohol into the intestine (chemical phase). The secretion following the introduction of the food into the intestine was also noted. First diminution and then complete cessation of gastric secretion occurred, on which 10 gm. of powdered yeast were added to the diet. In a few days a complete restoration of the secretory function of the gastric mucosa had taken place. An experiment on one animal is quoted below as representative.

One dog was placed on a diet of glucose, casein and olive oil, the vitamins which it had previously received (cod liver oil, yeast and tomato juice) being withheld. Diminution in the volume of the gastric secretion was the first objective evidence of vitamin deficiency. Eight days after the removal of the vitamins, while the animal was in its usual good spirits, 0.75 mg. of histamine injected subcutaneously provoked a secretion of only 4.6 cc. in 30 minutes compared with 56 cc. in the same time when vitamins were included in the diet. Sham-feeding likewise produced only 7 cc. in one hour compared with 76 cc. when the diet was adequate. Ten days later histamine again gave only 3.8 cc. and sham-feeding 2.2 cc. of juice with a large content of mucus in the same periods—this in spite of the previous introduction of 500 cc. of tap water to rule out any possibility of dehydration.

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[7] Farnum, M. B., *Arch. Int. Med.*, 1926, **37**, 42.

[8] Gilman, A., Dissertation, Yale University, 1931: "Chemical and physiological investigation on canine gastric secretion." Quoted from Cowgill, *J. Am. Med. Assn.*, June 18, 1932.

At the end of 3 weeks the animal was somewhat depressed, had liquid stools, but always retained a voracious appetite. 10 gm. of powdered yeast were added to the diet for 3 consecutive days. Tests now showed an almost complete restoration of the secretory function and the animal was once more in good spirits. 0.75 mg. of histamine injected subcutaneously now gave 48.5 cc. of gastric juice and sham-feeding 67.2 cc. in 45 minutes. Under histamine stimulation the free acidity rose to 0.409% and the total to 0.536% HCl, and the peptic power fell to 64 Mett's units. Sham-feeding produced a juice with free acidity from 0.365% to 0.431% and a total of from 0.523 to 0.555% HCl. The digestive power rose to 310 Mett's units.

The yeast was again withdrawn and after 6 days the secretagogue effect of histamine and sham-feeding was again almost abolished, yielding respectively 4.5 cc. and 10.6 cc. At the end of 12 days histamine stimulation produced only 1.0 cc. of thick mucus in 45 minutes.

The following day sham-feeding provoked no secretion whatever and 2 injections of 5% alcohol into the duodenum showed the stomach to be quite refractory apparently to all stimuli. There were only a few drops of thick mucus with no free acidity. The stomach had practically ceased to secrete. The dog soon after this appeared listless and developed a flaccid paralysis of the hind limbs, but retained its appetite. The hair was dry and came out in large quantities. The stools were partly formed, well coloured and occurred only once a day. The blood chemistry was normal, showing CO_2 combining power of 55.2 vol. %, NaCl 550 mg. %, serum calcium 10.6 mg. %, haematocrit reading 40%.

The experiments reported here indicate that the vitamins contained in yeast are necessary for the normal secretory activity of the gastric mucosa. No analyses have yet been made of the B fraction responsible for this effect. The complete achlorhydria under the condition of avitaminosis was obtained independently of anorexia and inanition, which never occurred in our experimental animals.

6903

Cultivation of Louping Ill Virus.

THOMAS M. RIVERS AND S. M. WARD.

From the Hospital of the Rockefeller Institute for Medical Research.

A simple method[1, 2] for the cultivation of vaccine virus was described which consisted of minced viable chick embryo tissue suspended either in Tyrode's solution or in a mixture of Tyrode's solution and rabbit serum. Recently it has been shown that the viruses of vesicular stomatitis[3] and poliomyelitis[4] are capable of multiplication in media similar to that used for vaccine virus. For the cultivation of the former active agent, minced chick embryo tissue suspended in Tyrode's solution was used, while for the latter minced chick embryo brain tissue suspended in a mixture of monkey serum (1 part) and Tyrode's solution (9 parts) was employed. During the past year we have been investigating louping ill, a neurotropic virus disease of sheep which in some respects resembles poliomyelitis. After the appearance of Gildemeister's note[4] on the cultivation of poliomyelitic virus, it seemed of interest to determine whether another neurotropic virus, the etiological agent of louping ill, is capable of growth *in vitro* under similar conditions.

Two types of media were used. The first (Series A) consisted of 0.1 gm. of minced chick embryo (11 days) brain suspended in a mixture (4.5 cc.) of monkey serum (1 part) and Tyrode's solution (9 parts). The second medium (Series B) was prepared in a manner similar to that of the first with the exception that chick

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[1] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[2] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

[3] Cox, H. R., Syverton, J. T., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 896.

[4] Gildemeister, E., *Cent. Bakt., Abt., 1, Ref.*, 1933, **109**, 284.

embryos from which the brains had been removed were used. The media, distributed in 4.5 cc. amounts in "collar flasks," were inoculated with 0.5 cc. of infectious material. The cultures were incubated 3 or 4 days at 37° C. Subcultures were made by means of the transfer of 0.5 cc. of the incubated cultures to flasks of freshly prepared medium. Thus at the time of each subculture a tenfold dilution of the virus occurred. The subcultures were tested from time to time to determine the presence of active virus. This was accomplished by means of the intracerebral inoculation (0.03 cc.) of mice with decimal dilutions (in Locke's solution) of the cultures. Infected mice became paralyzed in 5 to 7 days after inoculation and usually died.

Series A.—The cultures were initiated by the inoculation of 4.5 cc. of the medium with 0.5 cc. of a 10% emulsion of infectious mouse brain. Eleven subcultures have been made and the presence of virus was demonstrated by tests in mice in the second, fifth, and eighth. The virus in the eighth subculture was titrated and mice receiving 10^{-4} dilutions of the culture became paralyzed and died in a manner typical of louping ill. Inasmuch as the virus underwent a hundredfold dilution in the first culture and a tenfold dilution in subsequent ones, and since the mouse brain material from which the cultures were initiated was not infectious in dilutions greater than 10^{-6}, it is obvious that the virus multiplied in the cultures.

Series B.—The cultures were initiated by the inoculation of 4.5 cc. of the medium with 0.5 cc. of the fifth subculture in Series A. Six subcultures have been made and virus was shown to be present in the second and fourth. The virus in the fourth subculture was titrated and mice receiving 10^{-4} dilutions of the culture became paralyzed and died. In view of the dilution that occurred at the time subcultures were made, it is obvious that the virus also multiplied in cultures of Series B.

The media used for the cultivation of the viruses mentioned above are simple and easily prepared. It must be remembered, however, that living cells are essential and that any manipulation which leads to the death of all the cells renders the media valueless. Furthermore, care must be taken not to use too much tissue, because the viruses in the presence of an excess of tissue in the media will not multiply.

6904

Louping Ill in Man.

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From the Hospital of the Rockefeller Institute for Medical Research.

Inasmuch as louping ill,[1, 2] a natural disease of sheep, can be transmitted to monkeys[3] and mice,[4] and since the disease in these hosts in certain respects resembles poliomyelitis, we procured some of the virus for investigation. After we had been working with the active agent for 5 months, some of it was supplied to another laboratory in the Institute in which after a short time 3 of the workers, one after another, became sick. Two of the individuals developed what seemed to be influenza. In each instance this illness in the patient was followed by apparent health lasting a few days which in turn was followed by a definite encephalitis. The spinal fluids were sterile and showed a mononuclear pleocytosis and an increased amount of globulin. The third patient had what seemed to be influenza without symptoms of involvement of the central nervous system. Inquiry disclosed the fact that an English investigator also had an encephalitis more than a year ago after having worked with louping ill. In this case the spinal fluid also was sterile and showed an increased amount of globulin and a mononuclear pleocytosis. All of the patients made rapid and complete recoveries.

There is no record in the literature of the occurrence of louping ill in man, yet the disease was considered in connection with our cases and attempts to demonstrate the virus in the blood and spinal fluid of 2 of them were made without success. Failure to demonstrate the virus under such conditions, however, was not considered proof that the patients had not had louping ill. Consequently, inasmuch as we had developed a test[5] for the presence of neutralizing antibodies in the sera of monkeys immune to louping ill, we decided to apply the test to the sera of the 4 individuals who were suspected of having had the disease. In addition to these sera, we also tested the sera of other people working with the virus, as

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[1] Greig, J. R., Brownlee, A., Wilson, D. R., and Gordon, W. S., *Vet. Record*, 1931, **11**, 325.

[2] Pool, W. A., *Vet. J.*, 1931, **87**, 177, 239.

[3] Hurst, E. W., *J. Comp. Path. and Therap.*, 1931, **44**, 231.

[4] Allston, J. M., and Gibson, H. J., *Brit. J. Exp. Path.*, 1931, **12**, 82.

[5] Schwentker, F. F., Rivers, T. M., and Finkelstein, M. H., *J. Exp. Med.*, 1933, **57**, 955.

well as the sera of a number of individuals who had had no known contact with the active agent.

Seventy-seven satisfactory tests have been made on the sera of 60 individuals. The sera from the 4 patients and 1 laboratory worker, actively engaged in the investigations, definitely neutralized the virus of louping ill. Tests on the sera of these 5 individuals were repeated several times and from all, except one, 2 specimens of sera were examined. The worker not listed as a patient was not sufficiently sick at any time to remain away from the laboratory after the introduction of the virus. Sera from 10 active laboratory workers or helpers who probably came in contact with the virus contained no demonstrable neutralizing antibodies for the virus. Sera from 4 South Americans, 4 Chinese, 5 Negroes in Baltimore, 1 Australian, and 25 individuals of New York City who were either normal, convalescent from acute infectious diseases, or affected with chronic maladies also contained no demonstrable neutralizing antibodies for the virus. One serum from a Negro with obesity and pulmonary edema and one serum from a patient with nephritis yielded questionable results and are still under investigation. The sera tested were collected from both sexes and from individuals 1 year to 68 years old. The average age was approximately 30 to 40.

Of the 60 individuals investigated, 15 might have had contact with the virus. Of these 15, 5, all of whom had been in close contact with the inciting agent, possess sera that definitely neutralize the virus of louping ill. Of the 5, 3 had had encephalitis, 1 had been sick with an influenza-like disease, and 1 had not been consciously ill after initiation of work with the virus. Of the 45 people without histories of contact with the virus, 2 possess sera that give questionable results and are still being studied. The results of our work cannot be taken as proof that man is susceptible to louping ill, but they are sufficiently suggestive to warrant the recording of them in order that workers handling the virus may take precautions to protect themselves. Our findings do indicate, however, that individuals who come in close contact with the virus of louping ill may develop in their sera neutralizing antibodies against the active agent.

6905

Quantitative Estimation of Ash after Microincineration.*

GORDON H. SCOTT. (Introduced by E. V. Cowdry.)

From the Department of Anatomy, Washington University School of Medicine.

One of the things to be desired in the study of microincinerated sections is an accurate means of estimating the relative quantities of inorganic salts in various cells. Such a method would permit a quantitative comparison of tissues taken from animals subjected to experimental procedures with similar normal cells. Furthermore it would be possible to study such processes as normal growth and differentiation in a more exact manner than the microincineration technique has permitted. A quantitative method also has many advantages in the examination of pathologic tissues and comparable normal ones. The technique for quantitative photography of incinerated sections devised by Schultz-Brauns[1] has many points to commend it; but it is, in the final analysis, a procedure which depends on the visual judgment of the observer. It seemed advisable, therefore, to develop a method which would be free from this objection.

The nature and distribution of the inorganic salts in cells and tissues has been described.[2, 3] The appearance of the ashed preparations in darkfield illumination is that of hoar frost on a blackened surface. With this means of illumination only the ash of the section is revealed by light reflected from the surfaces of the individual particles of mineral. It is assumed, therefore, as the particles are approximately the same size and nature, that the more mineral present in the microscopic field the greater the quantity of light reflected into the eye of the observer. This assumption has been checked, in as far as possible, by examining colloidal solutions at different dilutions with the darkfield microscope with the result that given a constant source of light the intensity of the beam emerging from the ocular is roughly proportional to the number of particles in the microscopic field. Inspection of many hundreds of incinerated slides has shown the same to hold for the ash. Conse-

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* Aided by an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science.

[1] Schultz-Brauns, O., *Z. f. wissensch. Mikr.*, 1931, **48**, 161.

[2] Scott, G. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 48.

[6] Scott, G. H., *Am. J. Anat.*, in press.

quently a measurement of the intensity of the light reflected from the ash provides a means of estimating its quantity.

With the aid of Doctors L. A. DuBridge and L. Van Atta of the Department of Physics a photoelectric cell and a suitable amplifying circuit was devised for the particular condition required. Since the amount of light visible in any darkfield preparation is comparatively small the current generated by the photoelectric cell had to be increased about 40,000 times by the amplifier before it could be measured with a sensitive galvanometer. The details of the apparatus will be given in a forthcoming publication.

The procedure for the estimation of ash is as follows: Sections of standard thickness, 4 microns, are incinerated and prepared for examination in the routine manner. The areas selected for measurement are located in the darkfield and the microscope is placed in a specially built photomicrographic apparatus and the field focussed sharply on the ground glass. The particular part of the field desired is located in the center of the ground glass and the remaining portion covered with black paper impervious to light. When this has been done the ground glass is replaced with clear glass and the light coming through the aperture of the paper covering is focussed by means of a plano-convex lens onto the sensitive plate of the photoelectric cell. The amount of current generated is then determined by a galvanometer reading. Thus the ash residue of any particular set of tissues or cells can be compared with that of another group.

Thus far the method does not permit an expression of the quantity of residue in terms of grams of any one substance or even of total ash. However, it does give a measure of the salt present on a relative basis. For example, it has been possible to compare on a percentage basis the amount of ash in incinerated sections of blood vessels taken from normal monkeys with those of monkeys which have been fed varying amounts of irradiated ergosterol. In this instance the normal is used as a base line and the experimental tissues recorded as a percentage of the normal. The large cells found in the nervous system are particularly adapted to study in this way. The salt changes in the intestinal epithelium during active absorption are also susceptible to investigation. The normal (fasting) intestine was used as 100% and the ash of the actively functioning epithelium measured as a percentage of it.

6906

Clinical Study of Bone Marrow by the Method of Sternal Puncture.

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From the St. Louis University Medical School.

The material was secured by sternal puncture by a technique similar to that of Arinkin.[1] More than 50 cases were used representing a wide variety of clinical conditions. Smears made from the marrow material were stained with Wright's stain, Cresyl blue reticulocyte stain, Giemsa stain and peroxidase stains, no variations in technique being made from that of peripheral blood smears. This report is limited to a discussion of the Wright stained preparations.

The marrow cells are well-preserved and stained. Differential counts of the marrow cell elements have been made. The average of 7 cases with a normal peripheral blood picture was the following cells: Myeloblasts 2.4%; myelocytes 7%; Jung Kernige 6.7%; Stag Kernige 14%; II Segment Neutrophiles 10%; III Segment Neutrophiles 6.3%; IV, V, etc., Segment Neutrophiles 1.1%; Eosinophiles 1%; Basophiles 0.3%; Monocytes and Reticulo-endothelial elements 9%; Lymphoid Cells 24.9%; Megaloblasts 5.2%; Normoblasts 6.9%; Primitive Cells 2.6%. These findings agree rather closely with those of Arinkin[1] on similar types of cases.

The high percentage of immature granulocytes in our counts confirms the concept of Arneth and Schilling that the neutrophilic nucleus changes progressively with age from the round to the segmented form. The high percentage of juveniles and stabs confirms the idea that these are "young" granulocytes.

Cells indistinguishable by Wright's stain from the lymphocytes of peripheral blood are present in practically all of our bone marrow preparations. These we have designated as lymphoid cells. Certain small primitive cells and young myeloblasts[2] may be included in this group. The percentage of lymphoid cells is definitely greater than would be expected from the possible contamination with lymphocytes of peripheral blood. We have not attempted a separation of lymphoblasts from myeloblasts in the Wright stain preparations—all are termed myeloblasts except in the case of lymphatic leukemia. Mitotic divisions of cells in the bone marrow smears is largely limited to an intensely basophilic cell which varies in morphology both from the typical myeloblast and the typical megaloblast. It is a larger cell than those we have termed "Lymphoid cells". This cell we have called the primitive cell in our differential counts. Occasional mitotic division is seen in hemoglobin-containing erythroblasts.

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[1] Arinkin, M. J., *Klin. Med.*, 1929, **7**, 135.

[2] Isaacs, Raphael, *Arch. Int. Med.*, Dec., 1932.

Schilling[3] classes the appearance of megaloblasts in bone marrow as hyper-regeneration and as indicative of failure of normal regeneration. Our counts show large numbers of typical megaloblasts in all stages of development. In no instance are they completely absent. The megaloblastic marrow of the untreated cases of pernicious anemia is in our series marked by an increase in the primitive cells and very early megaloblasts rather than by an increase of the more mature hemoglobin-containing forms. Myeloblasts 6.5%; Myelocytes 9.6%; Jung Kernige 6.5%; Stab Kernige 10.0%; II Segment Neutrophiles 7.5%; III Segment Neutrophiles 3.0%; IV, V, etc., Segment Neutrophiles 1.5%; Eosinophiles 2.5%; Monocytes and Reticulo-endothelial elements 16%; Lymphoid cells 21.5%; Megaloblasts 7.5%; Normoblasts 8.0%; Primitive cells 7.0%.

The marrow of lymphatic leukemia shows marked alteration from the normal picture in that abnormal lymphocytic cells far outnumber all other cell types. The following differential illustrates this point: Lymphocytes 4.5%; Lymphoblasts 90.0%; Megaloblasts 2.5%; Normoblasts 1.0%; Primitive cells 2.0%.

The marrow of myeloid leukemia shows an increase of myeloblasts and myelocytes: Myeloblasts 14.0%; Myelocytes 11.0%; Jung Kernige 14.0%; Stag Kernige 12.0%; II Segment Neutrophiles 11.0%; III Segment Neutrophiles 5.0%; IV, V, etc., Segment Neutrophiles 1.5%; Eosinophiles 2.0%; Basophiles 1.0%; Monocytes and Reticulo-endothelial elements 5.0%; Lymphocytes 10.0%; Normoblasts 1.0%; Primitive cells 1.5%.

One atypical leukemia shows a mixed marrow picture with many lymphocytes and erythroblasts: Myeloblasts 5.0%; Myelocytes 13.5%; Jung Kernige 1.5%; II Segment Neutrophiles 3.5%; III Segment Neutrophiles 0.5%; Monocytes and Reticulo-endothelial elements 0.5%; Lymphocytes 30.0%; Megaloblasts 6.5%; Normoblasts 27.5%; Primitive cells 8.5%.

A single case of aplastic anemia showed a marked decrease in erythroblasts and granulocytic cells but on the other hand showed a marked increase in primitive cells, myeloblasts and lymphocytes: Myeloblasts 22.0%; Myelocytes 7.0%; Jung Kernige 1.0%; Stab Kernige 1.0%; II Segment Neutrophiles 3.0%; Monocytes and Reticulo-endothelial elements 4.0%; Lymphocytes 42.0%; Megaloblasts 2.0%; Normoblasts 1.0%; Primitive cells 17.0%.

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[3] Schilling, Victor, The Blood Picture, 106. (Translated by R. B. H. Gradwohl.)

It is obvious that the method of sternal puncture secures a specimen from only one small part of the extensive marrow system. Hence it is not surprising that variations seen by examining sections from many portions of the marrow may not be reflected in the sternal specimen. However, when the marrow is altered throughout its extent, alterations in the sternal marrow are to be expected.

The sternal puncture method is excellent for comparison of peripheral blood and marrow cell morphology. For clinical diagnosis it will only occasionally be useful.

6907

Effect of Urea upon Activity of Bacteriophage.*

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In attempting to minimize denaturation of phage by heat we employed a number of substances which various investigators have found to prevent denaturation of proteins.[1] While some of these substances delayed denaturation of phage (saccharose, glycerine and to lesser extent $CaCl_2$ or glucose) other substances (sodium salicylate, Bayer 205, or urea) exhibited no gross protective effect. During these experiments it was noted, however, that when mixtures containing phage and urea were plated with homologous bacteria at intervals during the experiment (before the inactivation of phage has occurred) the plaques of lysis appeared to be larger and noticeably clearer than on control plates containing the phage without the addition of urea. This apparent intensification of lysis in the presence of urea suggested the possibility that addition of urea may perhaps bring out the lytic activity of phage under circumstances where lysis is ordinarily inhibited, as for instance on 4%

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* This work was supported from a grant by the Rockefeller Foundation to Washington University for research in science.

[1] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 802.

agar. As we have shown, under these circumstances bacteria failed to undergo lysis, although the phage effect is not entirely abolished, as evidenced by the increase in the rate of multiplication of bacteria in contact with it.[2] We were interested in determining whether the addition of urea to the medium would promote lysis under these circumstances.

To measured amounts of a suspension of bacteria (growth from 10 agar slants of an 18 hour culture suspended in 40 cc. of broth) were added different amounts of a sterile (filtered) 50% solution of urea as shown in the protocol; 1.5 cc. of the respective mixtures were added each to 10 cc. of molten agar of different concentrations, and after thorough mixing poured into Petri plates, each mixture was made in duplicate. After a complete solidification of agar (15-30 minutes), a series of small drops (about 2 mm. in diameter) of bacteriophage (titer 10^{-6} cc.) was deposited on the surface of each plate by means of a fine capillary. As a control, similar drops of plain broth were also placed on each plate and incubated overnight at 37° C.

Next morning the plates were examined to determine the extent of lysis of bacteria in contact with droplets of phage. Findings illustrated in the protocol represent such an experiment in which *B. coli* and a corresponding bacteriophage were used. It will be noted that:

1. By itself, presence of urea in the medium did not cause any marked change in the growth of *B. coli* until the concentration of urea reached 6%, when it inhibited the growth. (columns 9 to 13).

2. In the absence of urea (columns 1 and 2) the intensity of lysis decreased as the concentration of agar increased. In the plates with agar concentration of 4% and 5%, the lysis was completely absent; moreover, the growth of bacteria on the spots where phage was deposited was much heavier than on the surrounding medium,[2] (E. G. = excess growth) while the spots on which broth was placed (control) showed an even growth throughout. (0 = no change.)

3. Addition of urea to the medium intensified the lysis (columns 3-8) in proportion to the concentration of urea present. This was indicated on 2% and 3% agar by the greater size and clearness of lysed areas as compared with controls (columns 1 and 2); while on 4% or 5% agar a fair degree of lysis was secured in the presence

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[2] Bronfenbrenner, J., and Hetler, D. M., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 480, and 1932, **29**, 806.

TABLE I.
Effect of Urea upon Lysis of *B. Coli.*

Column No.		1	2	3	4	5	6	7	8	9	10	11	12	13
50% urea added in cc.		—	—	.6	.6	.8	.8	1.0	1.0	—	.6	.8	1.0	1.2
H_2O added in cc.		1.0	1.0	.4	.4	.2	.2	—	—	1.0	.4	.2	—	—
B. coli suspension*		.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5	.5
Final concentration of urea, %		—	—	3	3	4	4	5	5	—	3	4	5	6
		Resulting mixtures added each to 10 cc. of molten agar of different concentration and poured into Petri plates												
		Drops of phage** and of broth respectively deposited on the surface of agar								Inhibition of growth by concentrated agar and by urea (control)				
1% agar	phage	++++	++++	++++	++++	++++	++++	++++	++++	heavy	heavy	heavy	good	poor
	broth	O	O	O	O	O	O	O	O	growth	growth	growth	growth	growth
2 "	phage	++	++	+++	+++	+++	++++	++++	++++	heavy	heavy	good	good	poor
	broth	O	O	O	O	O	O	O	O	growth	growth	growth	growth	growth
3 "	phage	+	EG	+	+	++	++	++	+++	heavy	good	good	good	poor
	broth	O	O	O	O	O	O	O	O	growth	growth	growth	growth	growth
4 "	phage	EG	EG	O	+	+	+	++	++	good	fair	fair	fair	poor
	broth	O	O	O	O	O	O	O	O	growth	growth	growth	growth	growth
5 "	phage	EG	EG	EG	EG	EG	O	++	++	good	fair	fair	fair	poor
	broth	O	O	O	O	O	O	O	O	growth	growth	growth	growth	growth

* Bacterial growth from 10 slants suspended in 40 cc. of broth.

** Phage titer 10^{-6} cc.

++++ = very marked lysis.

+++ = marked lysis.

++ = fair lysis

+ = weak "

EG = excess growth of bacteria under the drop of phage.

O = no change.

of 5% urea (columns 7 and 8), in corresponding plates in the absence of (columns 1 and 2) or with lower concentrations of urea (columns 3 to 6) only trace or no lysis occurred.

4. Stained contact prints made by depositing glass cover slips directly upon the spots where phage was placed previously showed that wherever lysis took place, surviving organisms located in the immediate proximity of lysed areas were greatly swollen. On the contrary, contact prints from the spots where deposition of drops of phage failed to elicit lysis as well as from the spots where drops of plain broth were deposited (control) showed the organisms of normal size exclusively. For example, organisms were found to be normal in size under the drops of phage on 5% agar containing 4% urea (columns 5 and 6) while on 5% agar containing 5% of urea (columns 7 and 8) they were greatly swollen.

Experiments analogous to the one demonstrated here were repeated a number of times with various combinations of phage and bacteria. In general it was found possible to reproduce this phenomenon regularly with various Gram-negative bacteria. When Gram-positive bacteria were used, clear-cut results were obtained only with *B. megatherium.* With *B. diphtheriae* and with Staphylococcus these experiments were not successful on account of the inhibition of growth by urea even in the lowest concentration used. (3%).

In the earlier experiments we came to the conclusion that failure of lysis to take place on media containing high concentration of agar or gelatin is not due to the change in the susceptibility of bacteria to lysis nor to the inactivation of phage through the adsorption by the colloids of the medium, but to the competition for water between the medium and the bacteria.[3]

If this conclusion is valid, the present experiments suggest that addition of suitable concentrations of urea to the medium (5% agar) prevents the binding of water by the agar and thus permits the swelling and subsequent bursting of bacteria to take place.

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[3] Bronfenbrenner, J., Monograph on "Filterable Viruses." Edited by T. Rivers (Williams and Wilkins, 1927).

6908

Fiber Distribution in Optic and Saphenous Nerves.

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"Either the nerves themselves may communicate impressions different in quality to the sensorium, which in every instance remains the same; or else the vibrations of the nervous principle may in every nerve be the same, ... ", etc.[1]

"The results therefore fail to support the view (of Bishop and Heinbecker), that there are fiber types distinguishable by time to maximum, conduction rate, irritability and refractory period. . . . . Since it is possible to recognize differences in the physiological responses of individual axons, it is no longer incumbent upon physiologists to adhere to the doctrine of specific nerve energies."[2]

The latter quotation seems to present a third alternative to those recognized by Johannes Müller in the former, a formulation of his doctrine of specific nerve energies. Müller emphasized the specificity of the whole sensory pathway, being unable to decide whether the differences in sensory pathways were assignable to qualitative differences in the responses of the nerve fibers concerned, or to differences in their terminal connections. The possibility apparently never occurred to him that the *same fiber* mediated different sensations by means of *impulses* of *different character,* which appears to be the interpretation placed by Blair and Erlanger on the variations they observe to take place in the responses of one and the same nerve fiber. Other suggestions, such as that of Adrian, that repetitive response of a fiber to its sense organ is a factor in sensation, or that the pattern in which different fibers respond is significant, are only elaborations of Müller's hypothesis, and the possibility which Blair and Erlanger propose offers the first escape since medieval philosophers debated the question in an experimental vacuum, to physiologists chafing at the restrictions of Müller's dictum.

Before availing oneself of this avenue of escape, however, one would like to know, in relation to nerves dissected out of the body, and subjected to experimental procedures of stimulating and recording by means of complicated mechanical arrangements, what fortuitous factors might themselves cause variations in the responses of individual axons. One might also wonder whether such variations as are observed in excised axons do in fact cause demonstrable variations in sensory effect in animals or man. The other differences between the experimental results of Bishop and Heinbecker and of Blair and Erlanger are quantitative ones, and if one reads "groups" in place of "types" in the objection cited above, these would be unimportant. But Blair and Erlanger's present hypothesis still fails to account for the finding that stimulation of the first group of axons in animal or human skin nerves causes sensations of touch, and stimulation of a quite different group of axons causes sensations of pain.[3]

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[1] Müller, Johannes, *Physiology,* 1844, (Engl. trans. 1848, 1059).

[2] Blair, E., and Erlanger, J., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 728.

Further evidence is now at hand, concerning the same structure that Müller used to illustrate his thesis, the optic tract. Heinbecker, Bishop and O'Leary, in various studies of sensory nerves [3, 4, 5], have pointed out correlations between the physiological properties of groups of axons and, on the one hand, the histological structure of these fibers, and on the other, the function served by them in the body. These sensory nerves have now been compared with the optic nerve. The latter in the frog exhibits 3 major groups of potentials separating out on conduction, with rates of 18, 4, and 0.5 meters per second respectively. In the rabbit, the optic nerve being recorded in the body with blood supply, the first 2 groups correspond, with rates of 50 and 20 meters per second respectively, and a third smaller group, at about 3 meters per second, appears not to correspond precisely with the third group in the frog. Comparing this situation with that in the mammalian saphenous nerve, where a first group of fibers mediates sensations of touch and joint and muscle sense, and a second mediates pain and temperature, the first group, at least, in the optic nerve, excites impulses that give rise to action currents in the optic cortex, and no sensation except vision is known to arise from optic nerve stimulation.

The physiological properties of the corresponding groups of fibers in the saphenous and optic nerves are quite similar, but the sensations aroused by their activities are presumably different. It would thus be improper to infer that one type of fiber always performed the same function everywhere in the body, and no such claim has been presented. On the other hand, the demonstration that stimulation of fibers with one set of properties has sensory effects determined mainly by their central connections, leaves the alternative proposed by Blair and Erlanger at best a subsidiary one, and leaves it possible for physiologists to adhere with some degree of fortitude to the doctrine of specific nerve energies.

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[3] Heinbecker, P., Bishop, G. H., and O'Leary, J., *Arch. of Neur. and Psychiat.*, 1933, **29**, 771.

[4] O'Leary, J., Heinbecker, P., and Bishop, G. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 302.

[5] Heinbecker, P., Bishop, G. H., and O'Leary, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 304.

6909

Asphyxia of the Frog's Kidneys.

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Urine formation was measured in normal frogs by repeatedly weighing individuals whose cloacae were bound for a known period of time and later unbound. It was observed that in the absence of oxygen no urine formed. Even when diuresis was favored, by subcutaneous injection of water or 1% urea solution, no urine reached the bladder. When the breathing was interrupted by appropriate pithing or anesthesia the same inhibition of urine formation occurred, provided the frogs were kept at temperatures (20° to 27° C.) high enough so that oxygen was not adequately supplied to the blood through the skin. After 2 hours of asphyxia, anuria persisted for 1 to 4 hours, until such time as the frogs resumed breathing. As the frogs recovered, extra urine was formed until the initial net body weight was reestablished. The heart's circulation was maintained throughout. Two alternatives presented themselves: did (glomerular) fluid cease to form, or was the fluid that formed completely reabsorbed (by the tubules)? If no fluid formed was the circulation inadequate, or was oxygen indispensable for the excretion of water?

The exposed kidneys were examined in pithed frogs that were lying in a closed gas chamber. Blood invariably ceased to flow through the glomeruli within 1 minute after deprivation of oxygen. Often the glomeruli emptied completely of blood, and the whole kidney became paler. No corpuscles and hence presumably no plasma flowed through the glomerular capillaries until 1 minute after oxygen was again supplied to them. A relatively low concentration of oxygen was adequate to maintain glomerular circulation in the exposed kidney. Portions of the kidneys other than the glomeruli continued in the absence of oxygen to receive a copious supply of blood, and it is believed that the asphyxia exerted its peculiar effect because of the special nature of the glomerular circulation.

6910

Variations in Calcification of Teeth as Shown by the Use of Grenz Rays.*

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CHARLES F. BODECKER.

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Dental decay may be closely related to disturbances in calcium-phosphorous metabolism. There has been little evidence to indicate the mechanism by which such systemic factors operate on the fully erupted tooth. One possibility in this connection is that the tooth can undergo post-eruptive changes in calcification in response to such systemic changes. To determine whether such intradental changes do occur, Bodecker and Applebaum[1] made roentgenological study of teeth in ground section, 1 mm. thick. The radiation was obtained from an X-ray machine which operates at about 40,000 volts. By this method, only gross differences in radiolucency were recognizable—such as that between dentin and enamel, or the partial decalcification associated with a lesion of incipient caries. In no case was it possible to distinguish any minute structural detail. Similar results have been obtained by Heiwinkel.[2]

Recently, Fricke[3] found that excellent radiographs of insects can be obtained by means of soft X-rays (Grenz or Boundary rays). He employed a specially constructed X-ray tube which operates at potentials as low as 4,000 volts. With the generous cooperation of Dr. Fricke, we were able to examine several thin sections of teeth with his apparatus. Since minute structural variations in teeth

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* Aided by a grant from the Commonwealth Fund.

[1] Bodecker, C. F., and Applebaum, E. A., *Dental Cosmos*, 1931, **73**, 995.

[2] Heiwinkel, M., *Vierteljahrsschrift für Zahnheilkunde*, 1932, **48**, 247.

[3] Fricke, H., *Radiography and Clinical Photography*, 1932, **8**, 12.

have never before been observed by this method, we report our technique.

Ground sections of teeth are prepared by means of a special planing machine.[4] The thickness of these sections is in the neighborhood of 0.15 mm.; for any one section it is constant to ± 0.01 mm., at least in the region of the crown. Such uniformity is greater than can be obtained by hand grinding. The section is mounted in Canada balsam, between 2 layers of black paper; this prevents its drying and generally facilitates its manipulation. The section thus mounted is placed in contact with the X-ray plate in a suitable holder and an exposure is made. We now use plates with a fine grained emulsion, especially prepared by Eastman Kodak Company. These have the advantage of permitting of microscopic examination of the radiographs at a magnification of 20 X, without disturbance from the appearance of the emulsion grains. Thus it is possible to distinguish much detail that would otherwise not be discernible.

In general, the method is so sensitive to variations in radiopacity, that a difference in thickness of no more than 0.03 mm. may result in a complete over-exposure in one portion of a section, whereas another part may yield a normal picture. Even a well-planed section will require a series of exposures under different conditions of voltage, milliamperage, and time in order to yield a complete radiographic record. This is necessary because of the great difference in radiopacity of dentin and enamel, and also because of any marked differences in calcification which may obtain between adjacent portions of the section.

Some of the structures in human teeth which have been found to be associated with variations in radiopacity are as follows: bands of Schreger, early stages of secondary decay of enamel, enamel spindles, contour lines of Owen, interglobular spaces, and striated variations in the dentin which are parallel with the dentinal tubules. In addition, a number of other phenomena have been observed, which are not so well characterized; e. g., a thin enamel crack or lamella which contains a highly radiopaque filling (possibly due to "healing"), a fan-shaped radiolucency that starts at a narrow region in the roof of the pulp chamber and radiates across the dentin and into the enamel, a radiolucent zone in the dentin surrounding the pulp chamber but at a slight distance from its margin, and well defined variations in the partially decalcified enamel of incipient caries lesions.

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[4] Bodecker, C. F., *Dental Cosmos,* 1926, **68**, 860.

6911

Can a Dye Base Penetrate into Living Cells from a Relatively Strongly Basic Dye Solution?

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The color of azure B begins to alter from blue to violet at about pH 10 (the more the higher the pH) until at about pH 13 it turns red. These color changes are instantaneous and readily reversible on appropriately lowering the pH values; they are not progressive on standing for 3 hours. They are due to tautomeric change from the dissociated blue dye salt to the undissociated red dye base. The half-way point in the color change from blue to red occurs roughly in the neighborhood of pH 12. These results show that azure B is a relatively strong base existing chiefly in the form of salt in the pH range available for living cells of *Nitella* (roughly between pH 6 and pH 9).

On shaking the azure B solution at pH 9.4 and pH 8.5 with carbon tetrachloride the red dye base is extracted by the latter, the more the higher the pH value. The ratio of the apparent absorption coefficients[1] is roughly (K_{ap} at pH 9.4)$\div$(K_{ap} at pH 8.5)$=\frac{1.3}{0.15}=8.7$. This ratio agrees with the ratio (percent of dye base at pH 9.4)$\div$(percent of dye base at pH 8.5 $= 2.44 \div 0.31 = 8$, when the basic dissociation constant of the dye is $K_b = 10^{-3}$. Such a ratio at higher pH values might show whether the dissociation constant is $K_b = 10^{-3}$ or higher, but owing to the color change this determination is not possible by this method. These results show that azure B is strongly basic but the dye base has a high absorption coefficient between carbon tetrachloride and water so that it can be extracted on shaking the mixture in spite of its low percentage in the aqueous phase at these pH values.

The rate of penetration of azure B at these pH values into the vacuoles of artificial[1] cells (with carbon tetrachloride as the membrane) as well as into living cells of *Nitella* increases with rise in the pH values. The rate represents the reciprocal of time it took for the dye to reach a given concentration in the vacuolar sap. The ratio is (rate at pH 9.4)$\div$(rate at pH 8.5) $= 3$, for the artificial cell and for the living cell. This ratio is lower than the ratio obtained for the apparent absorption coefficients. These results show that the rate is a function of the absorption coefficient but the function is not a linear one. The concentrations employed are dilute (below 0.0006 M) at which the concentrations are expected to be about equal to activities.

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[1] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 993.

The azure B which penetrates the vacuole is capable of raising the pH value of the sap, and has the same absorption spectrum as the azure B outside.

The importance of these results is in showing that if the dye base has a high enough absorption coefficient and if it is capable of being changed into the dye salt by the sap, it will pass into the acid vacuolar sap rapidly in spite of the fact that the dye is strongly basic. These results support the author's theory of multiple absorption coefficients.[1]

6912

Influence of Carbon Dioxide upon Hydrogen Ion Concentration of Frog's Skeletal Muscle.

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The anterior tibial muscles of *Rana pipiens* were removed, sprinkled with powdered quinhydrone, and mounted between a platinum plate electrode and an agar-KCl bridge connected with a saturated KCl-calomel half-cell.[1] The muscle and electrodes were inserted in a glass chamber through which known gas mixtures could be flushed. The potential differences were read in the usual way by means of a Type K potentiometer and galvanometer.

After the initial rapid acid shift, muscle macerated with quinhydrone slowly became more and more acid in 5% CO_2 in O_2. Intact muscle in 5% CO_2 in O_2 shifts rapidly acid in the first few minutes' exposure. This reaction is followed by a small alkaline drift, succeeded by a plateau which is maintained for 20 to 40 minutes. The secondary alkaline drift is attributed to the mobilization of base which is presumably a less rapid process than the inward diffusion of CO_2. The plateau usually terminated in an acid drift.

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* Aided by a grant from the Hendricks Research Fund, Syracuse University.

[1] Benson, C. C., *J. Biol. Chem.*, 1928, **78**, 583.

If the plateau values are assumed to be the pH of the tissue at definite CO_2 tensions and if these are compared with the hydrogen ion concentrations calculated from CO_2 combining power data,[2] the agreement is found to be good between 36 and 100 mm. Hg. CO_2 tensions. Above this range the electrometric method gives readings 0.1 to 0.2 pH higher. Below 36 mm. Hg. CO_2 tension, plateau values were not obtained in one hour.

In the presence of tank N_2 or tank O_2 the pH of resting muscle became progressively more acid at a decreasingly rapid rate. Apparently at alkaline reactions (pH 7.3 to 8.0) pure O_2 cannot prevent acid production, although the tissue reaction is maintained at a constant level in the presence of 5% CO_2 in O_2. The acid shift in the presence of O_2 could be delayed and markedly decreased although it is not absent in muscles poisoned with iodoacetic acid. The lactic acid relations of these muscles are being investigated.

In the presence of tank CO_2 the initial rapid acid shift was succeeded by a prolonged alkaline drift which is probably due to phosphagen destruction.[3]

Muscles exposed to 150 mm. Hg. CO_2 tension for 5 hours showed a more alkaline plateau than the matched muscles exposed to the same CO_2 tension for 1 hour. This is in agreement with the CO_2 dissociation data obtained in this laboratory.[2]

Up to at least 45 minutes the pH of muscles in CO_2/O_2 gas mixtures could usually be reversed to or near the original level by flushing O_2 through the chamber.

6913

Nuclear Configuration of Thalamus of Macacus Rhesus.

RICHARD L. CROUCH. (Introduced by Edgar Allen.)

From the Anatomy Department, University of Missouri School of Medicine.

The thalamic nuclei of the rat, armadillo, Tupaia, Carnivora, *Lemur catta,* and Cercopithecus have been identified from cell stained preparations by various authors. These studies are of interest because they form a basis for a comparative study of the thalamus, because a knowledge of the nuclei is necessary for an accurate study of the connections of the thalamus with other portions of the nervous system, and because a knowledge of both the nuclei and fiber connections is necessary for carrying out and interpreting experimental work on the thalamus. For these three reasons the present investigation has been made.

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[2] Root, W. S., *J. Cell. and Comp. Physiol.*, 1933, **3**, 101.

[3] Lipmann, F., and Meyerhof, O., *Biochem. Z.*, 1930, **227**, 84.

Five monkey brains were fixed in trichloracetic acid and stained with toluidine blue, according to the technic of Huber.[1] Two were sectioned frontally, 2 transversely, and one sagittally. The cell groupings and arrangements, their separation by fiber masses, and outstanding differences in cell types were used as criteria for designating nuclei.

The nuclei were divided into anterior, middle, midline, lateral, ventral, and posterior groups. The anterior group consists of the anteromedial, anterodorsal, and anteroventral nuclei. They resemble the 3 anterior nuclei of lower mammals, with the exception of the anterodorsal nucleus, which is markedly reduced in size. The reduction of the anterodorsal nucleus is gradual from the lower mammals to the higher.

The nuclei of the medial group are parataenialis medialis and lateralis, medialis dorsalis, medialis ventralis, parafascicularis, centre median, paracentralis, and centralis lateralis. The parataenial nuclei and the nucleus medialis ventralis are much smaller than they are in the lower mammals, while the nucleus medialis dorsalis and the centre median have increased a great deal. The other nuclei of the medial group are very similar to those of Carnivora.

The midline nuclei are interparataenialis, interanteromedialis, centralis medialis, paraventricularis anterior and posterior, and an undifferentiated area termed the central gray mass. This whole region is very small and it is not nearly so well developed as it is in Carnivora.

The lateral region is composed of the nuclei lateralis dorsalis, lateralis anteromedialis, lateralis anterolateralis, lateralis posterior, angularis, and reticularis. This region increases in both size and complexity through phylogeny. The increased number of nuclei and their different arrangement in *Macacus rhesus* make it impossible to compare them with the lateral nuclei of Carnivora and Tarsius, but they can be homologized with the lateral nuclei of *Lemur catta* and Cercopithecus.

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[1] Huber, G. C., 1927. Contributions to medical science dedicated to Aldred Scott Warthin. Wohr., Ann Arbor.

The nuclei ventralis anterolateralis, anteromedialis, intermedius, posterolateralis, posteromedialis, and posteroinferior make up the ventral group. This group increases in size and complexity in phylogeny just as the lateral group does. At the present time these nuclei can be compared to only the ventral nuclei of other primates.

The pulvinar makes up most of the posterior region. It is divided into medial, lateral, and inferior nuclei, the first 2 of which can be subdivided into smaller portions. The other 2 nuclei of the posterior region are the nucleus limitans and nucleus suprageniculatus, which are homologous to the similarly named nuclei in Carnivora and in other primates. The pulvinar seems to represent the posterior nucleus, the posterior portion of the lateral nucleus, and the pretectal nucleus of lower mammals.

Homologies of the lateral, ventral, and posterior groups cannot be definitely made until the fiber connections of these nuclei are determined.

6914

Nuclear Configuration of Subthalamus and Hypothalamus of Macacus Rhesus.

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From the Anatomy Department, University of Missouri School of Medicine.

This work is a phase of the general program of study on the diencephalon. The same technic and method of study were used here as were described in the preceding paper.

The preoptic area is so closely associated with the hypothalamus that its nuclei were also studied. The following nuclei were identified: medial, and lateral preoptic nuclei, nucleus paraventricularis preopticus, and nucleus interstitialis pedunculi thalami. These all appear to be homologous to the nuclei of the same name described by Rioch[1] for Carnivora.

The nuclei of the hypothalamus can be arbitrarily divided into 2 main groups, the nuclei of the infundibulum, and those of the mammillary system. The infundibular group will be considered first. Lying along the floor of the infundibulum and above the chiasma are the nuclei ovoideus, tangentialis, and supraopticus difussus. The nuclei lying close to or around the hypothalamic ventricle, are the nucleus filiformis, and the nuclei hypothalamicus paraventricularis ventralis, dorsalis, and posterior. The nuclei hypothalamicus anterior and parvocellularis lie in the anterior region of the infundibulum and just behind these nuclei are the nuclei hypothalamicus dorsalis, dorsomedialis, ventromedialis, and nucleus perifornicalis. In the posterior portion of the infundibulum, anterior to the mammillary region are the posterior hypothalamic nucleus and the nucleus hypothalamicus ventro-lateralis. Lateral to all of these nuclei and extending nearly the whole length of the infundibulum is the lateral hypothalamic nucleus.

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[1] Rioch, D. McK., *J. Comp. Neurol.*, 1929, **49**, 1.

The principal nucleus of the mammillary region is the medial mammillary nucleus. Surrounding this nucleus are the premammillary, inframammillary, supramammillary, and lateral mammillary nuclei. Just lateral to this group is a well defined nucleus, the nucleus intercalatus.

In the subthalamus were found the entopeduncular nucleus, zona incerta, nucleus subthalamicus, Fields H_1 and H_2 of Forel, and the substantia nigra.

Both the subthalamic and the hypothalamic regions of *Macacus rhesus* resemble very closely these areas of Carnivora, rat, and other lower mammals.

6915

Ovarian Hormone and Traumatic Stimulation of Monkey's Cervix to a Condition Resembling Early Cancer.

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The ovarian follicular hormone has been shown by Allen[1, 2] to be a powerful stimulator of epithelial growth in the female genital tract and breast. Large amounts of this hormone have been reported in the blood of cancer patients, both male and female.[3] Also

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[*] This work has been assisted by a grant to Dr. Edgar Allen from the Committee for Research in Problems of Sex of the National Research Council.

[1] Allen, Edgar, Contributions to Embryology, No. 98, 1927. Carnegie Inst. Wash. Pub. No. 380, 1.

[2] Allen, Edgar, Sex and Internal Secretions, 1932, The Williams and Wilkins Co., Baltimore.

[3] Dingemanse, E., Freud, J., de Jongh, S. E., and Laqueur, Ernest, *Arch. f. Gynäk.*, 1930, **141**, 225.

cancer tissue from men has been found to have a high content of theelin.[4] Lacassagne[5] gave theelin injections to 3 male mice from a strain in which 72% of the females developed spontaneous mammary cancer. The males of this strain normally never developed mammary cancer but the theelin injections produced mammary cancer in 5 to 6 months in each of the 3 males. Removal of the ovaries in mammary cancer strains of mice and rats markedly decreases or entirely prevents the development of spontaneous mammary cancer.[6, 7, 8] Hofbauer[9] reported that anterior hypophysis transplants and extracts in guinea pigs produced a precancerous condition in the cervix. His anterior pituitary treatment should have increased the production of the follicular hormone, although he considers that the pituitary hormone caused a direct stimulation of the cervical epithelium. These facts led us to attempt to produce cancer by injections of genital growth hormones combined with chronic trauma.[10]

Nine female *Macacus rhesus* monkeys were used. All except one were ovariectomized several months to 20 days before the beginning of the experiment. The cervices were traumatized by scissor cuts through a vaginal speculum. This procedure was repeated at 7 to 10 day intervals throughout the experiment. In addition to the cuts a small metal clip was clamped on the cervix in some of the animals. Subcutaneous injections of hormone were made twice daily, morning and evening as a rule. The daily dose of ovarian follicular hormone, given as theelin or amniotin, ranged from 50 to 100 rat units. The theelin† preparation used contained 50 rat units per cc. Two preparations of amniotin‡ containing 200 and 50 rat units per cc. respectively were used.

Six ovariectomized animals (monkeys 3, 4, 6, 7, 9) received cervical trauma and injections of the ovarian follicular hormone ranging in total dosage from 1,535 to 5,490 rat units, over time periods ranging from 16 to 90 days. Three of these animals (monkeys 6,

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[4] Loewe, S., Raudenbusch, W., and Voss, H. E., *Biochem. Z.*, 1932, **249**, 443.

[5] Lacassagne, M. A., *Compt. rend. Acad. d. sc.*, 1932, **195**, 630.

[6] Lathrop, A. E. C., and Loeb, Leo, *J. Cancer Res.*, 1916, **1**, 1.

[7] Cori, Carl F., *J. Exp. Med.*, 1927, **45**, 983.

[8] Murray, William S., *J. Cancer Res.*, 1928, **12**, 18.

[9] Hofbauer, J., *Zentralbl. f. Gynäk.*, 1930, **54**, 2393.

[10] Overholser, Milton D., and Allen, Edgar, *Anat. Rec.*, 1933, **55**, No. 4 Suppl., 32.

† Furnished by Parke, Davis & Co. for experimental purposes.

‡ Furnished by E. R. Squibb & Sons for experimental purposes.

7, 9) also received injections of corpus luteum hormone[11] during the latter part of the theelin injections. One ovariectomized animal (monkey 11) received hormone injections only; 2,802.5 rat units of follicular hormone and 10.7 cc. of corpus luteum hormone in 51 days. This animal was killed 33 days after the cessation of all injections. One ovariectomized animal (monkey 10) received cervical trauma only for 86 days. One animal with ovaries intact (monkey 8) received cervical trauma only for 119 days.

We have described[10] the changes that occurred in the cervices of these monkeys but realizing that we might be over-enthusiastic, we submitted our slides to Dr. James Ewing of Cornell University whose ability as a diagnostician is so well established. His pathological report follows:

Monkey 11. (Fig. 1). "Much papillary gland overgrowth, much epidermization, much infection, many areas of distinctly atypical

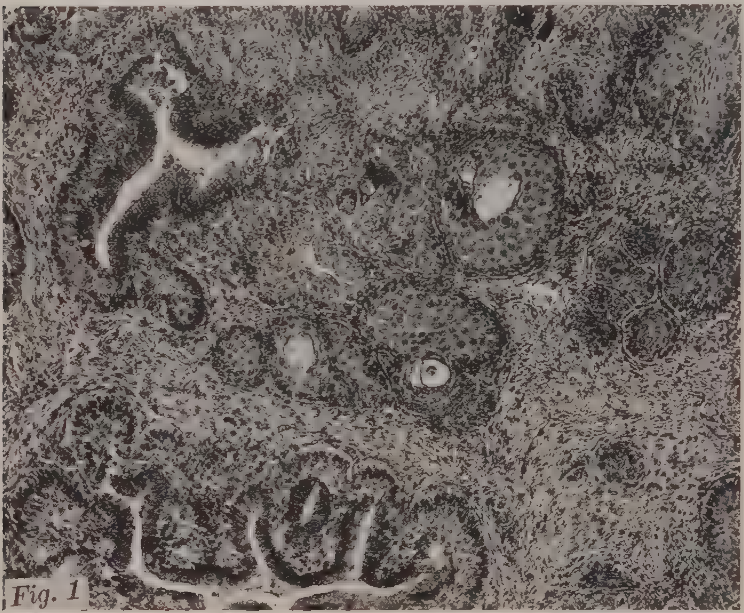

Fig. 1

Monkey 11. Section of cervix from ovariectomized animal that received only hormone injections. No cervical trauma given. Shows a close approximation to established cancer, especially the very atypical cells.

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[11] Prepared according to a method described by Turner, C. W., and Frank, A. H., *Mo. Agr. Exp. Sta. Res. Bul.*, 1932, **174**.

epidermoid cells. This is a close approximation to established cancer, especially the very atypical cells. I should have to pass the lesion as early but established cancer."

Monkey 9. (Fig. 2). "Shows early but essential features of infiltrating epidermoid carcinoma but in an ulcerating area. There is much infection which is probably an important factor." Another section from this same monkey "shows gland overgrowth, downgrowth, much epidermization, somewhat atypical, much infection. This is a pronounced precancerous lesion and trauma must be given an important place."

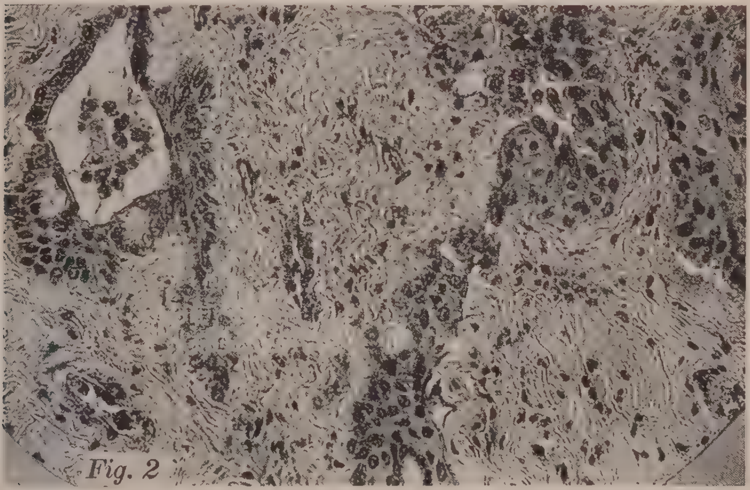

Fig. 2

Monkey 9. Section of cervix from ovariectomized animal that received cervical trauma and hormone injections. Shows early but essential features of infiltrating epidermoid carcinoma in an ulcerating area.

Monkey 10. Ovariectomized animal receiving trauma only: "Slight atypical downgrowth, slight infection."

Monkey 8. Normal animal receiving trauma only. Our own examination revealed a few areas of epidermization of the cervical glands.

A report on two semi-ovariectomized monkeys is included. They received cervical trauma and injections of follutein‡ (anterior pituitary like extract prepared from pregnancy urine). Dr. Ewing's report is as follows: *Monkey* 5. "Marked leukoplakia." *Monkey* 6-1. "Marked interstitial endocervicitis, slight precancerous changes."

Experiments are under way which attempt to further evaluate responsibility for the effects produced between hormone and traumatic stimulation.

6916

Pathogenicity of Serratia Marcescens (Bacillus Prodigiosus).

PAUL D. ROSAHN AND C. K. HU. (Introduced by L. Pearce.)

From the Laboratories of the Rockefeller Institute for Medical Research.

In connection with an investigation to determine the etiological agent responsible for a pandemic disease of rabbits[1], filtration experiments were conducted, and all filter candles were tested in the usual manner with a strain of *Serratia marcescens* (*Bacillus prodigiosus*) obtained from Institute stocks. As the work progressed, a fortuitous observation led to an investigation of the pathogenicity of this organism. The growth from a 24 hour agar slant was suspended in 10 cc. of Locke's solution, and rabbits and mice were injected with this material. Rabbits receiving 0.3 cc. intracerebrally, or 1.0 cc. intraperitoneally, intravenously, or intratesticularly, all died within 24 hours, and all mice injected with 0.6 cc. intraperitoneally or 0.05 cc. intracerebrally also died within 24 hours. A benign course attended the use of smaller amounts of the organism.

The gross findings at autopsy were striking. Hemorrhagic peritonitis with effusion, punctate hemorrhages in the adrenals, hemorrhagic lymph nodes, and small areas of focal necrosis in the liver were constant findings. An occasional animal had consolidated patches in the lungs. The spleen was usually large and swollen. *Bacillus prodigiosus* was recovered in pure culture from the brain, liver, spleen, lymph nodes, kidneys, lung, and blood.

A second strain of the organism was obtained from another laboratory. Morphologically and culturally it was identical with our own strain. When injected into animals in the large doses previously mentioned, it too caused death in less than 24 hours, and the same pathological picture was present.

Bertarelli[2] reported the death of rats, guinea pigs, and mice following the inoculation of massive doses of *Bacillus prodigiosus.* Our findings confirm Bertarelli's observations, and are of interest from two points of view, (1) that extensive hemorrhagic lesions and death of small laboratory animals follows the introduction of massive doses of an organism which in the ordinary sense is non-pathogenic. (2) When *Bacillus prodigiosus* is used as a test organism in filtration experiments, its presence in the inoculum after filtering through a leaky candle, might result in obscuring or even altering the experimental findings, since the organism itself has certain toxic properties.

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[1] Pearce, L., Rosahn, P. D., and Hu, C. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 894.

[2] Bertarelli, E., *Centr. Bakt., 1. Abt., Orig.*, 1903, **34**, 193, 312.

6917

Structural Changes in Anterior Horn Cells Following Central Lesions.

DONALD H. BARRON. (Introduced by J. S. Nicholas.)
From the Department of Anatomy, Albany Medical College.

The rôle of the impulses which impinge upon the cells of the "final common pathway" in maintaining their normal functional and structural character has not been fully appreciated. Warrington[1] demonstrated that after section of the dorsal roots of the post-thoracic cord segments, anterior horn cells on the same side became structurally altered, some of them ultimately degenerating. Cells on the contralateral side were also observed to be effected in certain cord segments, apparently those concerned with crossed reflexes. These observations did not include studies of the anterior horn cells after their dissociation from impulses descending to them from higher centers. The evidence so far accumulated has not shown whether or not motor horn cells in the spinal cord are effected directly after decortication or section of the pyramidal tracts, or trans-section of the spinal cord. The following experiments were performed with this question in mind.

In the first series of 17 rats, unilateral section of the corticospinal tract on the base of the medulla was performed. The observations on the functional disorganization of these rats will be reported later. In a second series (10 rats) the dorsal roots of the fifth, sixth, seventh, eighth cervical and the first thoracic segments

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[1] Warrington, W. B., *J. Physiol.*, 1898, **23**, 112.

were sectioned; one complete root was cut in each animal. In a third series of 4 rats, double trans-sections of the spinal cord were made, one at the level of the twelfth thoracic and the second at the level of the fourth lumbar vertebra. The dorsal root fibers of both sides entering this isolated segment of the cord were also cut. The vascular system was injured as little as possible. The animals of all 3 series were killed with ether between 15 and 20 days post-operatively.

Structural changes were observed in ventral horn cells only of the lower cervical segments of the animals in which one of the pyramids had been sectioned. This alteration was manifest by a swelling of the nucleus, and the collection of the chromatic material of the cytoplasm about it. In some instances, the nucleus had become definitely degenerate; in others, the nucleus had entirely disappeared and the cell was represented only by a hyaline mass. The altered cells in this first series were always few in number and confined solely to the side opposite the lesion.

Structurally altered and degenerate ventral horn cells were also found in the cords of those animals upon which rhizotomy had been performed. The degenerate cells were not confined to the segment supplied by the resected root but extended cranially and caudally into the adjoining segments. Cells on the contralateral side were also effected. They were located for the most part in the same segment.

The isolated segments of the cord, from which all of the segmental as well as descending impulses had been removed from the motor cells, showed complete degeneration. Both the motor horn and the internuncial cells were effected. Below the level of the second trans-section, the effected and degenerate cells were confined almost solely to the upper sacral segments. The mass of degenerating material represented by the ascending and descending fiber tracts may have been a mechanical factor which contributed to the extensive degeneration in this series.

These results demonstrate that following the section of dorsal roots or descending pathways of the spinal cord, anterior horn cells become structurally altered; some of them degenerate. In the rat, rhizotomy produces a much more extended degeneration than does a complete trans-section of the spinal cord in the lumbar region. The number of degenerate cells which appear following pyramidal section is not as large as that produced by cord trans-section. This fact may be associated with the limited function of the cortico-spinal tract in the rat.

These observations are quite in agreement with those of Warrington and are supported by the observations of Fulton and Keller[2] on the degeneration of peripheral nerves following trans-section of the spinal cord in monkeys.

6918

Observations on Effect of Repeated Administration of Nembutal in Guinea Pigs.

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The nembutal, sodium-ethyl (1-methyl-butyl) barbiturate, was dissolved in water and injected intraperitoneally into guinea pigs to see whether they had a tolerance for the drug. The animals were divided into 2 groups: (1) those receiving the drug twice weekly and (2) those receiving it daily.

The doses varied from 7.5 to 20 mg. per kilo body weight, but the dose for each pig remained constant. The minimum fatal dose (M.F.D.) was found to be 60 mg. per kilo. This amount killed 90% of the pigs.

To study the effects of the drug, the periods of time required for the development of 3 phases in the hypnotic state were selected: (1) the time from the injection until the animal could not move forward after painful stimulation (pinching); (2) the time from the injection until the animal could move forward after painful stimulation, and (3) the time from the injection until the animal could walk with a steady gait. The onset of sleep followed the first of these phases.

The weight of the animal greatly influenced the length of the hypnotic state, the heavier pig having the longer period of hypnosis, when the same size dose was given. There was, however, often considerable difference in the susceptibility of the individual animals which had approximately the same weight.

Injections of 25% of the M.F.D. of the drug induced sleep in about 65% of the animals. If this amount is given semi-weekly, the hypnotic effect, as judged by the failure of the onset of sleep, is greatly reduced within 4 weeks. The length of period of hypnosis is also markedly shortened. Daily doses of 25% of the M.F.D. produce the same effects in a much shorter time. Pigs which had received 16.6 to 20.8% of the M.F.D. semi-weekly for 6 weeks showed a definite tolerance to 29.1 to 33.3% of the M.F.D. Pigs which had had several semi-weekly injections of 25% M.F.D., or several daily injections of as much as 25% to 33% of the M.F.D., and which had shown definite tolerance in both groups as measured by the duration of sleep and hypnosis, were not protected against a dose of 60 mg. (M.F.D.) per kilo.

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[2] Fulton, J. F., and Keller, A. D., The Sign of Babinski, Springfield, 1932, 165.

We used 63 guinea pigs in the semi-weekly group. The results obtained from the first injection of the drug in each of the animals of this group served as controls. That is, animals which received subsequent doses were in an hypnotic state for a shorter time than they were after the first injection. Thus there were 63 controls for this group.

For the daily group 24 guinea pigs were employed. The results of the first injection in each pig served as controls, as above, so there were 24 controls for this group.

For determining the M. F. D. we sacrificed 24 normal animals.

To test the tolerance against a M. F. D. (60 mg. per kilo.) 33 pigs were used. These pigs had been treated previously as follows: (1), 4 pigs had received 19 semi-weekly injections, (2), 19 pigs had had 19 semi-weekly and 14 daily injections, and (3), 10 pigs had had 9 daily injections.

In all 111 individual guinea pigs were used.

6919

Effects of Different Anterior Pituitaries and Human Pregnancy Urine on Rat Sex Organs.*

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

We have shown[1, 2] that anterior pituitaries of different species as

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 642, 1128. *Endocrinology*, 1932, **16**, 129.

[2] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 741.

well as urine of pregnant women act differently on the sex organs of the guinea pig. The anterior pituitary of rat, rabbit, cat and guinea pig caused enlargement and maturation of follicles, formation of pseudo-corpora lutea and interstitial gland, and in some cases induced an ovulation. Injection of extracts as well as inoculation of pieces of anterior pituitary of cattle, hog and sheep produced an atresia of follicles so intense that it could not be attributed merely to a loss in weight of the animals; furthermore it gave rise to the formation of rudimentary interstitial gland and of usually small pseudocorpora lutea, which were essentially derived from hypertrophying theca interna and connective tissue elements, although in some cases a limited participation of granulosa could not be excluded. In exceptional cases we observed apparently an ovulation at very early stages following administration of this kind of anterior pituitary. Urine of pregnant women resembled in its action the anterior pituitaries of rabbit and rat, but differed from them in that it did not produce large mature follicles in the ovaries of the injected animals.

Since, as a test animal for the presence of anterior pituitary-like substances, the rat is commonly used, we now have studied the action of anterior pituitary of different species and of the urine of pregnant women on rats, to determine how far the results obtained were similar to those obtained in the guinea pig. For the most part young rats, weighing between 30 and 40 gm., but in some cases older animals, with initial weights of approximately 50 and 75 gm., were used. As in our former investigations, we studied microscopically in each case the ovaries in serial sections and parts of the other organs. More than 70 rats were used. We here present a summary of the results.

1. Daily inoculations for a variable number of days, of one-half rabbit anterior pituitary, of one or 2 rat anterior pituitaries and injections of one to 2 cc. of urine of pregnant women acted approximately in the same manner. They caused an enlargement of the interstitial gland, which developed as a result of the hypertrophy of the theca interna in atretic small follicles. In contradistinction to the condition found in the guinea pig, where, in addition to theca interna apparently also ordinary connective tissue cells situated around medullary vessels may change into interstitial gland, in the rat this change was limited to the theca interna cells. Also, in some cases, there was enlargement of the theca interna around certain growing follicles.

There was an increase in the number of follicles which developed into large follicles and also of large follicles the granulosa of which showed maturation, as manifested by a slight increase in the amount of cytoplasm. However, in the rat the maturation changes in the granulosa are much less evident than in the guinea pig. In certain cases when the follicles have reached this stage of development, the capillaries in the theca interna enlarge, become engorged with blood, and invade the granulosa. A layer of connective tissue begins to line the follicular cavity. The granulosa cells now enlarge and become pseudo-lutein cells, and these may divide mitotically. The egg in the meantime has undergone maturation divisions and subsequently further changes take place. These pseudolutein bodies become gradually converted into pseudocorpora lutea, as a result of organization of the central cavity by vessels and connective tissue, which later contracts. The pseudolutein tissue may further enlarge. This is the typical process which occurs in a great number of follicles. In many of these pseudolutein bodies and pseudocorpora lutea, remnants of eggs can be found, but not in every case. I have not seen any definite evidence of ovulation under these conditions, but I do not as yet wish to deny that it may sometimes occur.

The vessels in the medulla of the ovary are usually enlarged, and there may develop an edematous condition in the ovary which may lead to injury of the interstitial gland and even of growing follicles. Thus, gradually, the degeneration of granulosa of such follicles may become quite prominent, when the stage of full development of many pseudocorpora lutea has been reached. It is perhaps also due to this hyperemia that in full-sized follicles the intrafollicular fluid may increase considerably, dilate the follicles and exert a pressure on the granulosa.

The vagina, which in these cases usually begins to open on the third or fourth day of the experiment, undergoes proliferation and keratinization. Subsequently, the keratin is cast off and the squamous epithelium may be reduced in thickness. The uterine epithelium becomes usually high cylindrical at a certain stage and the mammary gland shows increased proliferation. These changes are more pronounced in rats injected with pregnancy urine, on account of the presence of follicular hormones in this urine.

2. Daily inoculation of one or 2 guinea pig anterior pituitaries shows effects much less marked in the ovary of the rat. We find merely growing follicles of various sizes—exclusive of large mature follicles—as well as follicles in various stages of degeneration.

After 8 days of inoculation we encounter follicles which seem to approach maturation. The vagina may show a limited proliferation, but does not usually form typical squamous epithelium. The uterus is in a resting state or shows only slight signs of stimulation. The mammary gland remains relatively inactive. We find then in the rat, as well as in the guinea pig, that the effects of guinea pig anterior pituitary are much less marked than those of rabbit and rat anterior pituitary. In the rat this difference between the effects of these types of anterior pituitaries seems to be perhaps still more pronounced than in the guinea pig. However, we have a right to assume that it is of a quantitative rather than of a qualitative character.

3. Daily injections into rats of quantities of acid extracts of anterior pituitary of cattle varying between 0.1 and 1 cc. do not lead to the development of large, mature follicles. The follicles seem on the whole to correspond to the average type of those observed in normal ovaries. There may be seen, however, some fairly large follicles. Granulosa degeneration of follicles of medium and somewhat larger size is usually prominent. From the fourth day on, pseudocorpora lutea appear which are mostly of small size, corresponding in character to hypertrophic atretic follicles, in the wall of which the cells in certain areas assume the character of pseudolutein cells. These pseudolutein cells are usually surrounded by capillaries. In some cases such pseudocorpora lutea are somewhat larger. One has the impression that one has to deal in these cases with a transformation of theca interna elements and of connective tissue cells in pseudolutein tissue, a process which occurs essentially during the atresia of follicles. However, in a few ovaries, examined 3 or 4 days following the beginning of injections, I could see an ingrowth of some capillaries from the theca interna into the granulosa in follicles in which the cavity was lined by a layer of connective tissue. This ingrowth was quite localized and often accompanied by degeneration of granulosa cells nearby. In view of these observations, which we intend to test further, we do not wish to exclude the possibility that granulosa cells may participate in the formation of some of these small pseudocorpora lutea.

The interstitial gland around quite atretic follicles in the medulla does not enlarge as it does in the ovaries of animals treated with rat or rabbit anterior pituitary or with pregnancy urine. The vagina does not change into typical squamous epithelium and shows at best only a very slight mitotic activity. In the large majority of cases, the vagina remained closed during the period of injections. Also the uterus is in a resting condition, or it may show signs of only a mild stimulation. The mammary gland is likewise mostly in a relatively resting condition or is slightly proliferating.

We see then in the rat, as in the guinea pig, a marked difference between the effects of extract of cattle anterior pituitary and the effects of rabbit and rat anterior pituitary or of pregnancy urine. The pseudolutein bodies differ in character, in the latter cases, very markedly from those found after injection of extract of cattle anterior pituitary and resemble the structures seen in corresponding cases in the guinea pig. The behavior of the vagina, uterus and mammary gland likewise corresponds to the findings in the guinea pig.

4. However, in the rat the atresia of follicles, which we find in the guinea pig after injection of extract of cattle anterior pituitary, is lacking, presumably due to a greater power of resistance to injurious influences possessed by the granulosa cells of the rat follicles as compared with those in the guinea pig ovary. This greater resistance in the rat is also indicated by the lack of degeneration of these granulosa cells at the time of ovulation. This characteristic feature enables the rat to have such a short sexual cycle and the ovulation of so many follicles at the beginning of this cycle. The greater sensitiveness of the granulosa cells in the guinea pig explains certain differences in the effect of pregnancy urine which are lacking in the rat. On the whole, the ovary of the guinea pig seems thus to be a finer reagent for the effects of anterior pituitary preparations than the more resistant ovary of the rat.

5. Moreover the rat as a whole seems to be more resistant to the action of anterior pituitary preparations than the guinea pig. We have noticed this greater resistance formerly in the case of the thyroid gland, and we notice it now in the case of the sex organs, where pro unit weight of the animal we applied greater quantities of anterior pituitary material in the rat than in the guinea pig, in order to obtain the typical effects. This difference in the responsiveness between rat and guinea pig is especially evident in the case of the inoculation of guinea pig anterior pituitary.

6920

Anterior Pituitary Hormones Acting on the Ovary and Differences in the Reactions in Different Species.*

LEO LOEB.

From the Department of Pathology, Washington University School of Medicine.

There has been much discussion in recent literature as to whether the anterior pituitary gland produces one or 2 kinds of hormones which act on the mammalian ovary, and through the ovary on the uterus, vagina and mammary gland, or also directly on the last named organ. Zondek[1] and others hold that there are 2 types of anterior pituitary hormones acting on the ovary, (1) the follicular growth hormone, responsible for the growth of follicles and (2) the luteinizing hormone causing the production of corpora lutea and of lutein tissue in general.

As previously indicated, the facts which we have established as well as other facts only partly published can be explained most satisfactorily if we assume that there are at least 2 types of anterior pituitary hormones which act on the ovary, one of which affects also the thyroid gland. These 2 hormones are as follows: (1) A hormone (1) which is responsible for the increase in size of the ovarian follicles and for the maturation processes which take place in the granulosa of follicles. This hormone counteracts the tendency of the granulosa to undergo degeneration, leads to the maturation of the granulosa primarily of the large sized follicles, but may also cause beginning or further advanced maturation of the granulosa of medium-sized and even smaller follicles. If no inhibiting mechanisms are at work, the production of large mature follicles leads in due time to ovulation and to the formation of true corpora lutea with the consequences which follow from the functioning of these organs. However, in many other cases, the large-sized mature follicles do not give rise to ovulation, but an ingrowth of capillaries and of connective tissue cells from the theca interna into the granulosa of such follicles; this is followed by the transformation of the granulosa cells into lutein cells and is usually also accompanied by an increase in the size of the theca interna cells. Especially in the rat these processes can be well recognized.

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* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Zondek, B., *D. Hormone d. Ovariums u. d. Hypophysen Vorderlappens.* Berlin, 1931.

Whether this hormone actually induces an accelerated cell multiplication in the granulosa of the growing follicle or whether it allows merely the production of many large follicles by inhibiting the premature degeneration of the granulosa and by increasing the size of the granulosa cells and the amount of liquor folliculi remains to be determined. These various possibilities have not been distinguished by various authors. As I have observed,[2] the maturation process as such, which takes place in the granulosa cells, tends to diminish and suppress the proliferative activity of the follicular granulosa.

(2) A second hormone (II), either preformed in the anterior pituitary or originating after removal of the gland from the animal, acts in the opposite manner on the granulosa of the follicles, inhibits its growth or accelerates the degeneration of the granulosa cells in species in which the granulosa cells are of a more labile character. It allows the transformation of theca interna into interstitial gland or pseudolutein tissue and of certain connective tissue cells into lutein-like cells during or following the process of atresia of follicles. Thus structures can be produced which show various kinds of intermediate conditions between atretic follicles and corpora lutea.

These two effects, (1) the inhibition of growth of follicles and of the tendency of the granulosa to undergo maturation, and (2) production of a hypertrophic type of atresia of follicles, leading to the formation of structures which if they reach a relatively large size may resemble corpora lutea, and the production of an often only rudimentary interstitial gland, due to the activity of the theca interna and possibly also of certain connective tissue cells, are combined and may therefore probably be attributed to the same pituitary hormone, although the first effect may occur without being followed by the second effect.

Some doubt remains in regard to several points. There is associated with the action of the hormone I, which causes enlargement and maturation of the granulosa, a marked development of interstitial gland around atretic follicles and in the medulla of the ovary. These effects are usually more marked than the corresponding ones connected with hormone II. Shall we ascribe these hypertrophic changes in both cases to the presence of the hormone II? Or do they represent a further development of the changes initiated by the hormone I?

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[2] Loeb, Leo, *J. Morphol.*, 1911, **22**, 37; *Virchow's Arch.*, 1911, **206**, 278.

Pending the outcome of these investigations we may provisionally characterize hormone I as responsible for the development of follicles to large size and for the maturation of the granulosa, and hormone II as inhibiting the growth of follicles and causing the degeneration of granulosa in species in which the granulosa cells are very labile and show little resistance to injurious factors; this hormone also leads to hypertrophic changes in the theca interna and in certain connective tissue cells, especially those which are active during atresia of follicles, although we cannot as yet be quite certain as to the possible relationship of these effects to hormones I and II. It seems probable that the anterior pituitaries of different species each contain both hormones, but that the proportions differ much in different species.

The luteinization of granulosa is associated with a process and is due to a hormone different from the process and hormone concerned with the luteinization which takes place during atresia of non-mature follicles. It is therefore necessary to distinguish between different processes which in the end may lead to the production either of corpora lutea or of lutein-like structures.

The hormone acting as stimulator of the thyroid gland can be independent of the follicular growth and maturation hormone I, but we have always found it associated with hormone II and the formation of interstitial gland and other pseudolutein structures, which we may tentatively attribute to hormone II. This thyroid effect appears after inoculation of anterior pituitaries relatively rich in hormone I (rat, rabbit, guinea pig), only if at the same time interstitial gland and other types of lutein-like tissue are produced. The interpretation of this fact will vary in accordance with our interpretation of the relations which exist between the follicular growth and maturation promoting hormone (I) and the production of lutein-like structures. If we assume the latter also to be a function of hormone I, but to appear only if hormone I is present in larger quantity, then we would have to conclude that the thyroid stimulating hormone occurs also in association with hormone I.

In addition to the character of the active hormone, the type of the soil on which the hormone acts determines the resulting effects. We have previously shown that the thyroid of the guinea pig is much more responsive to stimulation by anterior pituitary than the thyroid of the rat. We thus reproduce in the guinea pig but not in the rat all the essential characteristics of Graves' disease. Similarly the ovary responds in the guinea pig more readily to anterior pituitary hormones than in the rat, due to a considerable extent to a greater inertia and to a lessened sensitiveness on the part of the granulosa in the rat. These properties of the granulosa make possible the short duration of the sexual cycle in the rat.

On the other hand, there is some indication that the fully formed corpora lutea are less resistant in the rat than in the guinea pig. This is also a necessary requisite, if the sexual cycle shall have a short duration, because as I have shown previously[3] the corpus luteum inhibits ovulation. Probably this greater sensitiveness of the corpora lutea to unfavorable conditions and their greater tendency to degeneration in the rat, as compared with the guinea pig, makes impossible the long-continued preservation of the corpora lutea following complete hysterectomy in the rat, an effect which can be easily accomplished in the guinea pig.

A further variable factor modifies the response of the ovary to the anterior pituitary hormones, namely the immunity which develops after long-continued application of these hormones in thyroid as well as in ovary.[4] We have furthermore shown that this state of immunity does not depend upon the production of antibodies in the blood serum.

The effects which these ovarian changes have on uterus, vagina and mammary gland can be predicted from what we know of the characteristic effects of the follicular and corpus luteum hormones during the normal cycle and during pregnancy. The follicular hormone causes proliferation of vagina, hyperemia and marked epithelial hypertrophy and very slight proliferation in the connective tissue of the uterine mucosa, as well as growth in the mammary gland, while the corpus luteum hormone causes predecidual proliferation in the mucosa and sensitizes the mucosa in such a way that various kinds of stimuli can then produce placenta and placentomata. The corpus luteum also inhibits ovulation and proliferation of the vaginal epithelium.[5] We find corresponding effects, if follicles are stimulated or on the contrary depressed and lutein tissue is produced experimentally in the ovary. In particular, do we find after inoculation of cattle anterior pituitary a resting stage in the vagina of the guinea pig, while the uterus may under certain conditions present the typical picture of predecidual proliferation. On the other hand, the inhibiting effect of certain lutein-like formations produced in the ovary under the influence of rat, rabbit or guinea pig anterior pituitary allows, in many cases, only a partial proliferation of the vaginal epithelium, notwithstanding the presence of many fully developed mature follicles which otherwise should be expected to lead to keratinization of the vaginal epithelium.

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[3] Loeb, Leo, *D. med. Wochenschr.*, 1911, **37**, 17; *Biol. Bull.*, 1914, **27**, 1; Papanicolaou, G. N., *Anat. Rec.*, 1920, **18**, 251.

[4] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 172.

[5] Loeb, Leo, PROC. SOC. EXP. BIOL. AND MED., 1923, **20**, 441; *Am. J. Physiol.*, 1927, **83**, 202; Loeb, Leo, and Kountz, W. B., *Am. J. Physiol.*, 1928, **84**, 283.

6921

Diseased Rats Showing Increase of Giardia and a Septicemic Bacillus coli.

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From the Protozoology Laboratory, University of California.

During a recent series of experiments by the Department of Physiology of the University of California there appeared a very high rate of mortality in young rats. These rats, ranging in age from 3 to 8 weeks, were brought to us for protozoological examination. The symptoms were as follows: yellowish watery faeces; haemoglobinuria in some cases; anemia; and decided lack of muscular coordination. Twenty-three such rats were examined by us. They were on a *raw* milk diet.

At biopsy and autopsy the duodenum was quite inflamed, while the jejunum and ileum were frequently black with congested blood. The lungs were often hyperemic. The macroscopic appearance of the liver, spleen, and kidneys was normal.

Microscopic examination revealed the presence of a far greater number of *Giardia* than ever before observed by us in rats. They were most abundant between the limits of 6 and 20 inches below the stomach in the jejunum and ileum. They were so abundant that the microscopic field contained countless numbers of these protozoa, while tissue scrapings from the wall of the intestine revealed them massed on the surface of the cells, apparently feeding on tissue juices.

Potter[1] distinguishes 2 species of *Giardia* from the rat: *G. lamblia* and *G. muris*. The forms found in these rats were of the *G. lamblia* type in the majority of cases, occasionally accompanied by *G. muris*.

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[1] Potter, L. A., *Am. J. Hyg.*, 1928, **8**, 77.

Due, however, to the fact that the disease was of such an acute nature, death occurring in from 24 to 48 hours, a concurrent bacterial infection was suspected. Cultures were therefore made aseptically in lactose-fermentation tubes from heart, liver, and intestine of typically diseased rats with the thought that we might recover one of the paratyphoid group. The bladder was also removed aseptically and the urine streaked on an agar plate. From the heart cultures we recovered 4 strains of a gram-negative lactose-fermenting bacillus. Similar organisms were recovered from the bladder of 2 rats, the small intestine of 2 rats, and the liver of 2 rats. These were grown in pure culture and put through the following sugars: lactose, glucose, sucrose, maltose, mannite, and xylose. The Voges-Proskauer tests were negative, and the methyl red positive. There was no liquefaction of gelatin. As a final test they were found to ferment salicin, which is now considered to be a more reliable sugar than dulcite.

The 8 strains isolated in pure culture were therefore proved to be identical in all respects; and by conforming with the distinguishing biochemical reaction of *B. coli,* was proved to be an aberrant, septicemic strain of that organism.

At the same time a sample of the raw milk used in the diet of the rats at weaning had been streaked out on eosin-methylene-blue plates. A strain was isolated from the milk, which proved to be identical in all biochemical respects with the strains isolated from the rats.

Eight strains (2 from urine, 2 from heart, 2 from intestine, 1 from liver, and 1 from milk) were injected intraperitoneally into the 6-week-old rats from the colony in the Department of Zoology. They each received 1.0 cc. of a heavy saline suspension of a 24-hour culture.

The strains from the heart, urine, and milk killed these inoculated rats in less than 18 hours. The intestine was inflamed, but not congested. The 2 strains from the intestine produced death, but not until after 2-5 days. The intestine was inflamed but not congested. This delayed reaction might be correlated with the original isolation of these 2 strains from the intestine which is the normal habitat of *Bacillus coli.* Those strains producing a septicaemia by their abnormal habitat in the organs had a much more toxic effect upon intraperitoneal injection.

It is known that the optimum pH for *Giardia* in rats is 6.43-6.52.[2]

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[2] Kofoid, C. A., McNeil, E., and Bonestell, A. E., *Univ. Calif. Publi. Zool.,* 1933, **39**, 179.

Bacillus coli is an acid-producer and when present so abundantly apparently affords a habitat for *Giardia* which is more favorable than that of the normal rat. It is interesting to note that in the rat which lived 5 days the number of *Giardia* was markedly increased, while in those rats which lived 12-18 hours there was not sufficient time for any marked increase in *Giardia.*

6922

Evidence of Secretion of Posterior Pituitary into Cerebro-Spinal Fluid under Influence of Heat.

H. G. BARBOUR AND W. E. HAMBOURGER.

From the Department of Pharmacology and Toxicology, Yale University.

At the present time there is lack of agreement as to the existence of demonstrable amounts of posterior pituitary substance in the normal cerebro-spinal fluid and some are even inclined to doubt that the amount of this substance can be increased by physiological stimuli. The state of our knowledge was well summarized by Cushing.[1] Pituitary substance is said to be augmented by the administration of certain organic extracts and drugs, by emotional stimuli and direct electrical stimulation of the hypothalamus (Karplus and Peczenik[2]).

We have examined by the guinea pig uterus test the cerebro-spinal fluid of cats exposed to hot and cold baths respectively. The amount of Ringer's solution used was 3 cc. to which was added from 0.2 to 0.4 cc. of either cisternal or ventricular fluid. The bath temperatures were either 20°C. or between 40 and 41°C. The duration in each case was 30 minutes. In the case of the hot bath the sample of the cerebro-spinal fluid was taken before removal from the water.

From 5 cats samples of cisternal fluid were taken none of which showed any oxytocic activity in the normal unanesthetized cat. After exposure to cold baths there was only one doubtful instance of oxytocic action from the 5 animals. After the hot bath, however, the cisternal fluid from 3 of the 5 cats showed definite oxytocic action.

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[1] Cushing, H., *Proceed. Nat. Acad. Sci.*, 1931, **17**, 163.

[2] Karplus, I. P., and Peczenik, O., *Arch. ges. Physiol.*, 1930, **225**, 654.

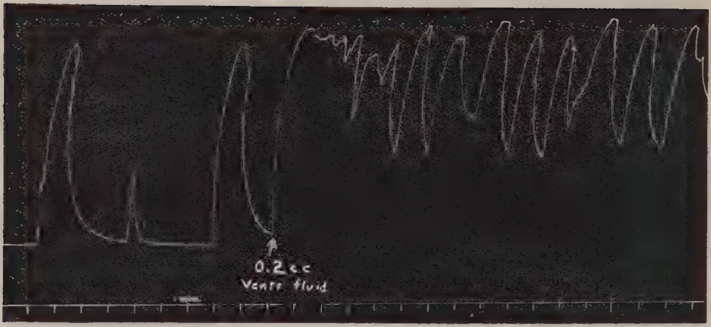

FIG. 1.
Effect upon isolated guinea pig uterus of 0.2 cc. ventricular fluid from cat after 30 minutes in bath at 40°C.

Two further cats were given 25 mg. per kilo of chloralose by stomach tube and the ventricular fluid tested. The fluid was removed through a small trephine hole just lateral to the sagittal and posterior to the coronal sutures. Again the fluid taken before the bath was found quite inactive but that removed after the 40° bath from both cats was decidedly more active than cisternal fluid.

Inasmuch as Solari[3] and others have shown that the hypophysectomized dog may preserve for many months a normal body temperature, it does not seem certain that posterior pituitary plays an essential rôle in heat regulation. Since, however, it has been shown in this laboratory[4] that pituitary, whether intraventricularly, intravenously or subcutaneously given, greatly dilutes the blood plasma, it may well be supposed that the substance affords an auxiliary physiological mechanism for promoting the dissipation of heat.

The amount of posterior pituitary substance secreted in the hot bath experiments was probably more than 10^{-3} International units.

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[3] Solari, L. A., *Comp. Rend. d. Soc. d. Biol.*, 1931, **108**, 125.

[4] Gilman, A., and Barbour, H. G., *J. Pharm. Exp. Therap.*, 1933, in press.

6923

Neutralization of Gastric Acidity Following Pyloric Closure.

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In previous experiments from this laboratory[1] it was shown that the neutralization of gastric acidity, studied by means of an acid test meal, was definitely accelerated by division of the pyloric sphincter. The present experiments show that closing the pylorus, partially or completely, produces the opposite effect, *i. e.,* a delay or complete absence of neutralization of the acid test meal.

Two series of experiments were performed. In one a silk ligature soaked in 25% $AgNO_3$ was tied around the pylorus so that occlusion short of complete obstruction was achieved. The only animals studied were those which remained in good condition and did not vomit following the operation (6 dogs). For periods as early as a few days and as late as several months a marked and sometimes complete delay in the neutralization of the acid test meal (200 cc. of 5% HCl fractionally aspirated) was found over the control curve obtained before operation. Preoperative curves were obtained in all but one of these dogs. In 5 other dogs operated on as controls no changes were noted.

In 7 dogs a pyloric snare was installed at operation. This could be closed or opened at will by means of a silver tube brought out through a separate stab wound. Several days or weeks after recovery the acid solution was introduced and it was possible in 8 successful experiments by tightening or loosening the snare to produce definite changes in the curve of neutralization. During periods when the pylorus was occluded the acid was neutralized incompletely or not at all; on its release the acidity dropped as promptly and rapidly as in the normal animal.

These experiments add further evidence to the idea previously expressed that there is an actual pyloric control of gastric acidity presumably because the sphincter influences the degree of regurgitation of alkaline duodenal contents particularly pancreatic juice into the stomach Of additional interest is the finding in many of our experiments of hyperaemia of the gastric mucosa and definite inflammatory changes in the duodenum most marked just distal to the partly occluded pylorus. In one dog a perforated ulcer at this point, with death from peritonitis, was found.

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[1] Elman, Robert, and Rowlette, A. P., *Arch. Surg.*, 1931, **22**, 426.

6924

Concentration of Calcium Ions in Biological Fluids.

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The sensitivity of the frog's heart to changes in the calcium content of a nutrient fluid has long been known[1, 2]. Utilization of this property for the estimation of calcium ions in biological fluids has, however, not been generally considered feasible, because considerable doubt has existed[3] as to whether the known sensitivity is to calcium ions or to total calcium. The method here presented is based upon (1) the demonstration, simultaneously reported, that the reaction of the frog's heart is specific for calcium ions, bound calcium being without effect, and (2) elimination or control of other factors which may influence the behavior of the heart.

The method consists essentially of recording the amplitude of the contraction of the frog's heart, suspended from a modified Straub cannula inserted through the aorta into the ventricle. After taking certain precautions to standardize the procedure, the concentration of calcium ions in the unknown solution or biological fluid is taken to be that of the known solution which produces an amplitude of contraction most nearly matching that produced by the unknown. A satisfactory preparation is sensitive to changes of 0.1 millimol per liter of calcium ions (0.4 mg. per 100 cc.) or less, so that the theoretical accuracy of the method is to within ± 0.1 mM per liter.

The method is applicable to such solutions or biological fluids as will sustain the action of the frog's heart without toxic effects. It can be used in artificially prepared salt solutions of widely varying composition, and has been found suitable for serum, plasma, cere-

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[1] Straub, W., *Verhandl. d. Gesellschaft deutscher Naturforscher und Aerzte,* 1912, **84**, 192.

[2] Trendelenburg, P., und Goebel, W., *Arch. f. exp. Path. u. Pharm,* 1921, **89**, 171.

[3] Klinke, K., *Ergebnisse der Physiol.,* 1928, **26**, 235,

brospinal fluid, chest fluid, and ascitic fluid of human origin, and for serum and cerebrospinal fluid of the dog. It has been found unnecessary to dilute these fluids, the only precaution being to make the known solutions isotonic with the unknowns, rather than with frog's blood.

The solutions used for comparison have been based upon the solution of van Dyke and Hastings[4] and made up to resemble as closely as possible the inorganic constituents of the fluids to be matched. It has been shown that with the frog's heart as indicator, variations of temperature, pH, magnesium, and phosphates within a rather wide range have little or no effect, and that a change of 1.0 mM in potassium concentration produces at the most less effect than a change of 0.1 mM in calcium concentration, so that it has been found possible to make up standard solutions approximating the average inorganic composition of the biological fluids to be studied, and in fact to omit magnesium and phosphates entirely from these standard solutions.

The only real difficulty with respect to biological fluids, and a possible source of error, is with respect to the presence or possible presence of pressor or depressor substances in these fluids, which may conceivably either mimic or mask the effect of calcium ions present in the fluid. Many of the biological fluids so far examined have contained a substance or substances increasing the amplitude of contraction of the heart, particularly if the heart is in a "hypodynamic" state. With fresh hearts this effect may not be observed, except when dog serum is used, this serum being rich in pressor substance.

In practice it is readily observed that the effects of pressor substances may easily be distinguished from the effects of calcium ions, chiefly by the rate of response of the heart to them, and by the rate of recovery after they are withdrawn. The response to changes in calcium ion concentration is an immediate one, the heart frequently being in equilibrium with a new solution by the time the change is completed. The effect of a pressor substance, however, is much more gradual, and persists for a much longer time after it is withdrawn. In practice, therefore, when the effect of a pressor substance is observed it is allowed to produce its maximum effect, and comparisons are then made at the new level just as though no pressor substances were present. That this method is valid has been shown by experiments in which adrenalin has been added to known solutions.

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[4] van Dyke, H. B., and Hastings, A. B., *Am. J. Physiol.*, 1928, **83**, 563.

Thus *recognizable* pressor effects have been eliminated as a complicating factor. We have not, however, up to the present, eliminated the possibility of other pressor or depressor substances having a speed of action and disappearance comparable to that of calcium ions. On the other hand, we have encountered no instances suggesting the presence of such substances.

Using the method as described the calcium ion concentration in certain biological fluids has been estimated. (Table 1).

TABLE I.
Estimations of Ca++ Concentration in Biological Fluids.

Fluid	Source	Diagnosis	Total Ca mM/L	Ca++ mM/L
C.S.F.	Human	Epilepsy	1.37	1.15
Serum	"	Normal	—	1.0
C.S.F.	"	No diagnosis	1.24	1.0
C.S.F.	Dog	Tetany	1.04	0.95
Serum	"	Normal	2.36	0.9
Chest	Human	Heart failure	2.11	0.8
Ascitic*	"	Pick's disease	3.00	1.05

*High protein content.

The concentration of calcium ions in the fluids studied was approximately 1.0 millimol per liter. From this, the non-ionized, diffusible calcium is estimated at not more than 0.25 millimol per liter in cerebrospinal fluid and, after allowance is made for the calcium bound by protein, not more than 0.5 millimol per liter in serum.

6925

Electrical and Mechanical Changes in Isolated Heart Following Changes in Calcium Content of Perfusing Fluid.

EMMET B. BAY, FRANKLIN C. MCLEAN AND A. BAIRD HASTINGS.

From the Department of Medicine, the Lasker Foundation for Medical Research, and the Physiological Laboratory, the University of Chicago.

The experiments here reported were performed on the isolated heart of the rabbit perfused at 38°C. by the method described by Locke, but without recirculation of fluids. Changes in amplitude of contraction were recorded on a drum, and electrical changes were recorded with a string galvanometer from one circuit only, the connections being made through non-polarizable electrodes to the aorta and to the apex.

Because of the known unphysiological nature of Locke's solution the solutions used for perfusion were based upon those described by van Dyke and Hastings, with the addition, in many instances, of sodium citrate and a higher total calcium concentration. In these solutions the calcium ion concentration has been calculated from the dissociation constant of calcium citrate as simultaneously reported. The solution used as the standard of reference usually contained 0.7 millimol of calcium ions per liter, and 0.8 millimol per liter of non-ionized calcium in the form of calcium citrate.

In solutions without citrate the amplitude of contraction of the heart varied directly with the amount of calcium in solution up to concentrations which produced irreversible changes. Omission of calcium from the perfusing fluid was followed by almost total cessation of contraction. Electrical impulses continued, as has been reported by others, although considerably modified, and recovery from these solutions was rapid. When, however, a small amount of citrate was added to the solutions containing no calcium all mechanical and electrical activity ceased and the heart did not recover in physiological solutions. Our interpretation in this case is that calcium ions are necessary for the continuation of electrical as well as mechanical activity, and that unless citrate is added to the perfusing fluid, sufficient calcium remains in the heart for electrical activity to continue.

With other conditions constant, a moderate reduction of Ca^{++} was followed by a prolongation of electrical systole, and usually by a reduction in the height of the T-wave. On the other hand, increase in Ca^{++} was followed by shortening of electrical systole, with no consistent change in the T-wave. A striking feature of all our observations, except under extreme conditions, was the disproportion between mechanical and electrical changes, the former being more marked. This is in contrast to the effects of potassium, which produces electrical changes disproportionately greater than mechanical changes.

6926

Fate of Acacia after Acacia-Saline Injections.

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Although acacia has been used extensively for intravenous injection in shock and more recently in nephrosis, its ultimate fate in the organism is unknown.

In the course of some experiments on the effect of repeated injections of gum acacia on rabbits, it was found that acacia disappeared from the blood after 3 to 4 days and that none appeared in the urine except in traces after administration of as much as 10 cc. of 30% solution. The following experiments were carried out to determine what had happened to it.

Two to 10 cc. of a 30% solution of acacia were injected into the heart of rabbits every third day until from 5 to 25 gm. had been given. The animals were killed and the kidney, spleen, liver, and a piece of leg muscle were removed for analysis. The tissues were weighed at once, minced, and a weighed sample was ground with sand. This was extracted with 20% trichloracetic acid followed by hot water, and the filtrates were combined in a volumetric flask. Acacia was determined in aliquot portions by the method of Youngburg.[1] The Youngburg method was checked with solutions of acacia and found to yield 13.5% furfural. This was the factor used in calculating the acacia values. The values for acacia in the liver ranged from 60 to 70%. The results in all experiments are probably plus or minus 10%. Determinations on control animals gave small blanks for pentose, possibly from nucleotides.[2] All pentose values were calculated to acacia for purposes of comparison.

In one of 5 strictly comparable experiments a 2 kg. rabbit was injected with 3-4 cc. of a 30% acacia solution every third day until 4.8 gm. had been given. From the analyses of tissues of this animal and of a control approximately the same weight (Table I) it ap-

TABLE I.

	Total Tissue (gm.)			Acacia Mg./Gm. Tissue			
	liver	spleen	kidney	liver	spleen	kidney	muscle
Control	48.7	2.0	16.0	10.4	6.6	3.5	3.5
No. 7	85.0	2.0	11.0	48.3	21.1	5.6	3.5

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[1] Youngburg, G. E., *J. Biol. Chem.*, 1927, **73**, 599.

[2] Kerr, S. E., and Blish, E. J., *J. Biol. Chem.*, 1932, **98**, 200.

pears that about 60% of the acacia was retained by the liver, a smaller proportion by the spleen, and much smaller amounts by kidney and muscle.

6927

On the Fatty Acids Essential in Nutrition.*

GEORGE O. BURR AND WILLIAM R. BROWN.

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Within the last few years a number of laboratories have published data in support of the view that diets quite low in fat are not complete. This is a reversal of the view held 10 years earlier. Working with diets extremely low in fats, Burr and Burr[1] showed that a disease supervened which could be cured by the addition of small amount of unsaturated fats or pure linolic acid. An examination of their data and that of other workers led to the postulation of essential fatty acids which could not be synthesized in adequate quantities by warm blooded animals. Their results have been repeated, partially or in full, by several workers. However, some workers [2, 3, 4, 5] have encountered scaly skin and necrotic tails in rats which were not curable by fat and this has led them to the view that we, too, are dealing not with a fat deficiency, but possibly with a vitamin B factor. There is no evidence that scaly skins and necrotic tails are specific for any single deficiency. Therefore, the production of these symptoms by one method does not preclude ready production by another.

Since all work in this laboratory strengthens the belief in beneficial effects of certain fats and fatty acids, it seems well at this

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* This work was supported by grants from the National Live Stock and Meat Board, from the Medical Research Fund and General Research Fund of the University of Minnesota.

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345. The diet is made of purified casein, sucrose, salt mixture, supplemented by concentrates of vitamins A, D and E fed on whole dried yeast (ether extracted) of proven high quality (Northwestern).

[2] Hume, E. M., and Smith, H. H., *Biochem. J.*, 1931, **25**, 300.

[3] Gregory, E., and Drummond, J. C., *Z. f. Vitaminforschung*, 1932, **1**, 257.

[4] Funk, C., Caspe, S., and Caspe, H., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 816.

[5] Roche, A., and Roche, J., *Comp. Rend. Soc. Biol. Paris*, 1932, **109**, 463.

time to point out differences in technique which may readily account for the lack of agreement.

The diets used by the above workers indicate they were all deficient in growth factors. Funk, Caspe and Caspe did not add adequate vitamin B. Roche and Roche found their pathological skins on diets so deficient that the rats weighed only 80-100 gm. after 12 weeks. Those rats receiving whole yeast instead of yeast extract showed no skin lesions. Hume and Smith state "The growth of the animals is not considered in the results; the supply of vitamins B_1 and B_2 was insufficient for good growth over the major part of the experiment so that growth performances have no significance." The vitamin B source was marmite. It should be noted further that their animals did not always receive enough vitamin A so that bladder stones were encountered. They also used starch diets in place of sugar diets. Gregory and Drummond likewise use an extract of yeast which obviously is greatly deficient in one or more of the water soluble vitamins. Gregory and Drummond's growth curve for a "fat-free" diet is reproduced as accurately as possible in Fig. 1. For comparison is given the average growth curve of a group of 24 rats recently raised in this laboratory and still under observation. The end of the 8-week growth period commonly used for vitamin B tests is marked by X. While the Gregory and Drummond rats grew 28 gm. in the 56 day period our rats grew 86 gm., or over 3 times as fast. The average growth of these female rats on our fat-free diet in our colony is, therefore, about 1.6 gm. daily. During the 4 weeks of most rapid growth preceding the onset of fat deficiency, they gained 2.1 gm. daily, which is considered by many workers normal for stock females. Since growth is the quantitative measure of vitamins B and G, our diets would be considered very high in both factors (which is to be expected when 0.65 gm. of high grade dried yeast is consumed daily) while the Gregory and Drummond diet VIIb would be rated as decidedly deficient in at least one of the growth factors.

Although we had earlier found additional yeast ineffective in improving our rats after they had begun to decline on the fat-free diets, this experiment was repeated on the entire group of 20 rats (4 having died). Their daily dose of yeast was increased from 0.65 gm. to 0.90 gm. daily (39% increase). Growth was not resumed. The decline in weight was not even checked. (Fig. 1). At the end of a 5 week period of increased yeast dosage, there was no general improvement of the skin and during this period 5 cases of severe hematuria appeared, one of which died. For comparison,

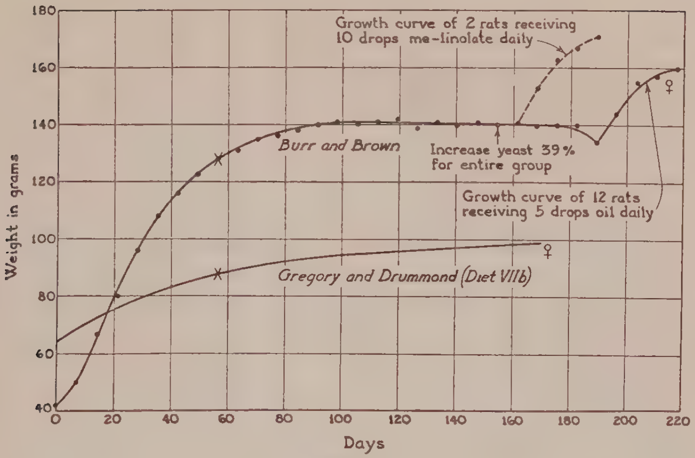

FIG. 1.

2 of the rats were fed 10 drops daily of pure methyl linolate at the same time. Their immediate growth response is shown as a branch of the main curve (Fig. 1).

As the animals were rapidly growing worse on the high yeast dose, fat supplements (5 drops daily) were now given to 12 of them (others used for analyses). The immediate growth response is seen in Fig. 1. At the end of 5 weeks a general improvement of the skin was evident. Hematuria disappeared from those rats which had shown it. No new cases appeared.

There is another important difference between the English work and ours. Hume and Smith find no scale or necrosis until the 180th day of experiment and Gregory and Drummond find it only after 90 days or more (2 cases out of 24 at 90 days). Rats on our diet show more or less severe scaliness throughout the groups within a few weeks. The scaliness appears during the period of rapid growth and could hardly be attributed to a lack of one of the growth factors.

It should be pointed out that fat deficiency is characterized by a late failure in growth (at 130-150 gm. weight) after an early period of rapid growth in the presence of excess growth factors. This failure is accompanied by a high percentage of kidney lesions,[6] abnormal gas exchange [7, 8] and abnormal water consumption.

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[6] Borland, V. G., and Jackson, C. M., *Arch. Path.*, 1931, **11**, 687.

[7] Wesson, L. G., and Burr, G. O., *J. Biol. Chem.*, 1931, **91**, 525.

[8] Burr, G. O., and Beber, A. J., *J. Biol. Chem.*, 1932, **97**, 36.

Finally, death always ensues after 6-10 months on the diet. But when our diet is supplemented with 10 drops daily of a good fat, animals remain in good health for 18 months and scaliness of the skin has never been encountered.

From the point of view of the general metabolism of the rat, scaliness of the skin may be of secondary importance. It occurs in varying degrees of severity from group to group and is difficult to measure quantitatively. Time of year seems to affect its severity and we suspect humidity as an important factor. It has been found that scale tends to disappear from the feet of rats kept for long periods of time in humid metabolism chambers. We are now checking this theory.

As pointed out in our earlier papers, tail necrosis and scaly skin can result from numerous dietary conditions. Some good examples are general underfeeding,[9] deficiency of G (B_2)[10], diets rich in egg white,[11] rancid fat in the diet,[12] and deficiency of fatty acids. It is not necessary (nor even justifiable without further work) to assume that these diverse causes work through a common factor such as abnormal skin lipids which we now believe responsible for the scaliness of our rats.

Conclusion. The recently reported cases of skin lesions discussed here were produced under conditions which indicate a lack of some growth factor. In no case were conditions such that uncomplicated fat-deficiency could result. It would be impossible, therefore, for these rats to respond to small doses of unsaturated fats. An adequate supply of all water soluble growth factors must be fed if the typical fat deficiency results are to be obtained. Growth should approximate that given by the daily consumption of 0.65 gm. or more of high grade dried yeast.

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[9] Smith, A. H., and Bogin, M., *Am. J. Path.*, 1927, **3**, 67.

[10] Goldberger, J., and Lilie, R. D., *Pub. Health Rep., U.S.P.H.S.*, 1926, **41**, 1025.

[11] Parsons, H. T., *J. Biol. Chem.*, 1931, **90**, 351.

[12] Whipple, D. V., Proc. Soc. Exp. Biol. and Med., 1932, **30**, 319.

6928

Innervation of Kidney of Toadfish.*

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From the Department of Anatomy, University of Minnesota.

While an intimate relation of nerve fibers to the epithelium of the renal tubules has been reported most strikingly by Smirnow,[1] the finer nervous mechanism of the kidney is still in doubt. The extrinsic nervous control of urinary secretion has been variously interpreted (Bieter,[2] Kuntz[3]), it being particularly difficult to separate results arising from vasomotor changes from strictly secretory phenomena. In the opinion of several investigators (for most recent summary of literature see Marshall and Grafflin[4]), at least the proximal convoluted segment of the renal tubule has important secretory functions. The aglomerular kidney of the toadfish *(Opsanus tau)* has afforded unusually favorable material for studying the secretory processes that may be carried on by the tubular epithelium since there are no glomeruli to complicate the process. The terminal portion consists only of a blind tubule with the histological characteristics of the proximal convoluted tubule of higher animals (Edwards[5]). Bieter[6] showed that in this animal the secretory pressure of urine as registered in the ureters can be greater than the blood pressure in the dorsal aorta. This phenomena together with the action of diuretics on the urinary output (Bieter[7]) and on the cytological structure of the tubular epithelium (Defrise[8]), and other related problems, naturally bring up the question of the nerve supply to these aglomerular tubules.

Dr. Bieter, having a supply of living toadfish for additional studies on the action of various drugs on kidney function, sug-

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* Aided by a grant from the research fund of the Graduate School, University of Minnesota.

[1] Smirnow, A. E., von, *Anat. Anz.*, 1901, **19**, 347.

[2] Bieter, R. N., *Am. J. Physiol.*, 1929, **91**, 436.

[3] Kuntz, A., Autonomic Nervous System, 1929 (Lea and Febiger, Philadelphia), 271.

[4] Marshall, E. K., Jr., and Grafflin, A. L., *J. Cell. and Comp. Physiol.*, 1932, **1**, 161.

[5] Edwards, J. G., *Anat. Rec.*, 1929, **44**, 15.

[6] Bieter, R. N., *Am. J. Physiol.*, 1931, **97**, 66.

[7] Bieter, R. N., *J. Pharm. and Exp. Ther.*, 1931, **43**, 399.

[8] Defrise, A., *Anat. Rec.*, 1932, **54**, 185.

gested this study and generously supplied an abundance of fresh material.

From a study of similar work on other animals, a variety of techniques were selected, including intra-vitam methylene blue in a buffer solution so that the pH could be changed as described by Hirt,[9] modified Bielschowsky stain (Oertel,[10] Rogers[11]), gold chloride, silver-pyridine (Ranson[12]), and various stains for connective tissue and muscle fibers.

The application of methylene blue and gold chloride to teased material was impractical because of the large amount of intertubular lymphoid tissue, so these methods were applied to small blocks of tissue which were afterward embedded in paraffin. Most of the methods gave very unreliable results due to the tendency for the argyrophil fibers of the reticular tissue to take the stain, in spite of the valuable suggestions of Beech and Davenport[13] with reference to the Bielschowsky methods. Some of these connective tissue fibers are very fine and closely resemble nerve fibers. The most specific staining of nerve fibers was obtained by Ranson's silver pyridine method.

The kidneys are in close contact with large spinal nerves and the sympathetic plexus. From the latter, numerous small nerves, composed of non-myelinated fibers, follow the blood vessels into the kidney. Numerous non-medullated nerve fibers also course in the wall of the ureters and terminate among the muscle fibers. Such fibers could be followed along the branches of the ureter as far as there is a muscle coat and considerable connective tissue, but not to the terminal portion where the wall is reduced to nothing but the epithelium and a basement membrane.

The nerves along the blood vessels and in the associated connective tissue are carried far into the kidney, the individual nerve fibers losing themselves among the muscle fibers of the vascular wall; but no nerves could be identified with certainty on or between the epithelial cells of adjacent urinary tubules where the reaction was very favorable for the staining of nerve fibers.

Hirt[9] comments on the difficulties encountered in bringing out the nerve terminals in kidney tubules of the frog and concluded that they were not nearly as numerous as one would expect from the work of Smirnow.[1] While this failure to find nerve fibers in the kidney epithelium may be due to inadequate technique, the fact that the very finest nerve fibers to smooth muscle within the kidney (in the ureter and blood vessels) could be readily seen in Ranson's silver-pyridine material, while adjacent tubular epithelium showed none, strongly indicates a lack of strictly secretory fibers to the kidney of the toadfish. There are, however, abundant vasomotor nerve fibers and nerve fibers to the musculature of the ureter and its branches.

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[9] Hirt, A., *Z. f. Anat. u. Entwcklngsges.*, 1930, **91**, 580.

[10] Oertel, H., *J. Path. and Bact.*, 1929, **32**, 558.

[11] Rogers, W. M., *Anat. Rec.*, 1931, **49**, 82.

[12] Ranson, S. W., *Am. J. Anat.*, 1911, **12**, 69; *J. Comp. Neur.*, 1912, **22**, 159.

[13] Beech, R. H., and Davenport, H. A., *Stain Techn.*, 1933, **8**, 13.

6929

Staining of the Adrenals with Neutral Silver Nitrate as a Test for Scurvy.

R. H. HAMILTON, JR. (Introduced by J. F. McClendon.)

From the Laboratory of Physiological Chemistry, University of Minnesota Medical School.

Moore and Ray[1] and Miller, Siehrs, and Brazda[2] observed that, whereas immersion of the cut surface of the adrenal gland from a normal guinea pig in neutral silver nitrate solution resulted in a blackening of the surface of the gland, there was no reduction by adrenal glands taken from scorbutic guinea pigs. Siehrs and Miller[3] showed that staining ability of the adrenals disappeared after the animal had been on a scorbutic diet for 6 days, but reappeared even after 18 days on a scorbutic diet if the animal were then fed 3 cc. of orange juice per day until the twenty-second day. They suggest that "the reduction test is a convenient qualitative method for testing for scurvy and for antiscorbutic substances."

The adrenals of 14 guinea pigs dying of scurvy were tested by immersion in neutral 0.4% silver nitrate solution for 30 minutes. None showed any staining. One animal fed on a scorbutic diet for 37 days was then given 1.5 cc. lemon juice daily for 4 days and was killed with ether. Its adrenals showed no staining, even after 30 minutes in silver nitrate.

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[1] Moore, T., and Ray, S. N., *Nature*, 1932, **130**, 997.

[2] Miller, C. O., Siehrs, A. E., and Brazda, F. G., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 636.

[3] Siehrs, A. E., and Miller, C. O., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 696.

Four guinea pigs that had been fed on the basal scorbutic diet plus 1.5 cc. of lemon juice daily were killed respectively on the 50th, 56th, 56th, and 57th days of the diet, and a piece of sliced adrenal from each was immersed in the silver nitrate solution for 30 minutes. At the end of this time there was in every case either no change in the color of the gland, or at most only a faint graying. In one case one whole gland and half of the other were ground with sand for a moment, water was mixed in, and the solid particles were centrifuged out. After the addition of silver nitrate solution to the extract there was no change in the color of the mixture in 30 minutes.

Under identical treatment with neutral 0.4% silver nitrate, the cut adrenal of a guinea pig that had been fed on oats, carrots, and alfalfa hay stained quite black within 15 minutes. A water extract of the other gland began to turn dark within 4 minutes after the addition of silver nitrate.

The 4 animals on the basal diet plus lemon juice were examined carefully for signs of scurvy. Their growth curves were normal. They showed no clinical signs of scurvy. Upon autopsy no hemorrhages were found in the muscles or in the wrist joints. The costochondral junctions were clean and sharp. None of the teeth were loose. No microscopic examinations were made.

Apparently it is necessary for the animal to have in its body an excess of vitamin C over and above its immediate requirements before any can be stored in the adrenal glands. If this hypothesis is true, one could not rely upon lack of staining of the adrenals with neutral silver nitrate as a qualitative test for the presence of scurvy sufficiently severe to produce gross signs. However, it might still be possible to use the reaction for assaying foods quantitatively for vitamin C. Animals could be maintained before the assay at such a level that the adrenals just failed to react. Upon feeding graded amounts of the test food to a series of animals, a positive reaction might be expected to appear within a few days in those receiving more vitamin C than is required for maintenance.

6930

A Biologic Assay of Blood and Feces of Infants Receiving Various Antirachitics.

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From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

It has been shown that the standard method of estimating and establishing the unitage of antirachitic agents by means of biologic assays on rats, does not conform to clinical experience.[1] For example, 40 units of irradiated milk, in other words 650 cc. daily, suffice to protect an infant from rickets whereas approximately 600 units of irradiated ergosterol (viosterol) or 240 units of cod liver oil are required to accomplish a similar result.

In an investigation of this problem an attempt was made to ascertain the antirachitic potency of the blood and the feces of infants who had been given these and other specific antirachitics. It was found possible to carry out satisfactory titrations of the blood when as little as 0.1 cc. of citrated blood was fed daily, or 0.8 for the period of 8 days in the course of the regular 10-day test. Usually twice this amount was fed and in some instances a total of 3.2 cc. of blood. Even less may be used if serum or plasma is fed. In an extended series of assays conducted in this way it developed that the relatively high potency of, for example, irradiated milk as compared to viosterol, could not be accounted for by the relative amounts of antirachitic factor in the blood. When protective doses were given, notwithstanding the fact that the clinical effect was about the same, a definitely larger number of units were found in the blood of the infants which had received viosterol. Evidently antirachitic effect clinically does not parallel the antirachitic content of the blood, as tested on rats. Possibly the explanation of the difference in activity when, for example, irradiated milk or irradiated ergosterol are given, can be ascribed to the production of dissimilar forms of the vitamin.

Assays of the feces served to confirm these results. Although differences in regard to absorption were found, viosterol, cod liver oil, irradiated milk, "yeast milk" were all well absorbed from the intestine. In general, the absorption was relatively the same; where the antirachitic unitage was large, more was recovered from the feces, and where a small number of units was fed the absolute amount in the feces was small. Direct irradiation of the infant resulted in a moderate amount of antirachitic in the blood and no excretion of vitamin in the feces.

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[1] Hess, A. F., and Lewis, J. M., *J. Am. Med. Assn.*, 1932, **99**, 647.

6931

Comparative Analysis of Forms of Calcium and Inorganic Phosphorus in Human and Cow's Milk.

ALFRED F. HESS AND HELEN RIVKIN BENJAMIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

It is a common observation that rickets occurs less frequently in breast-fed infants than in those which are artificially fed. The antirachitic superiority of human milk is not due to a greater content of vitamin D.[1] Moreover, cow's milk contains more Ca and P than does breast milk and the ratio of Ca:P is lower in the former. It was thought that, in spite of its lower content of total Ca and P, human milk might contain a larger amount of the most effective forms of these elements and that therein might lie an explanation for the difference in antirachitic value of the 2 kinds of milk.

In a recent communication,[2] a technic was described which rendered it possible to separate the Ca of serum into 4 forms and the P into 2. The technic involved removal, by means of dry $BaSO_4$, of the adsorbable forms of Ca and of inorganic P from the serum and from the corresponding ultrafiltrate. It was shown[3] further that one of the forms, namely the filtrable, adsorbable Ca-P complex, was the one primarily involved in bone formation. Gyorgy,[4] as well as others, have suggested that the Ca and P of milk exist in the form of complex salts and Klinke[5] has demonstrated that more than half of the Ca of cow's milk can be adsorbed with $Al(OH)_3$. There is, thus, some indication that milk may contain a fraction similar to the filtrable, adsorbable Ca-P complex of serum. Accordingly, the above mentioned technic was applied to a study of the forms of the Ca and inorganic P in human and in cow's milk. Irradiated cow's milk was also included in the investigation because of its increasing importance as an antirachitic agent.

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[1] Hess, A. F., and Weinstock, M., *Am. J. Dis. Child.*, 1927, **34**, 845. Outhouse, J., Macy, I. G., and Brekke, V., *J. Biol. Chem.*, 1928, **78**, 129.

[2] Benjamin, H. R., and Hess, A. F., *J. Biol. Chem.*, 1933, **100**, 27.

[3] Benjamin, H. R., *J. Biol. Chem.*, 1933, **100**, 57.

[4] Gyorgy, P., *Biochem. Z.*, 1923, **142**, 1.

[5] Klinke, K., *Ergeb. d. Physiol.*, 1928, **26**, 235.

It should be stated at once that, as regards its forms of Ca and P, milk represents a more complicated system than serum. It has been shown,[6] for example, that casein is capable of adsorbing Ca added to milk and the possibility has been raised[7] that some of the Ca and P normally present in milk is adsorbed to casein. The presence of citrates has also been suggested (Klinke) as a factor in the formation of Ca complexes in milk. The fractions separated from milk by means of $BaSO_4$ adsorption and ultrafiltration are, therefore, probably not single entities and the results are presented, not as indicating the complete partition of Ca and inorganic P but rather as a comparative analysis of different kinds of milk.

Method. The methods used in this study were those described for serum, with the following exceptions. For the adsorption test the milk or ultrafiltrate was diluted so as to contain about 10 mg. % of Ca. With human milk it was found that the same results were obtained when undiluted milk was used. For the determination of inorganic P the Fiske-Subbarow method[8] was used. The results were compared to those obtained by the use of a titrimetric method and found to be identical. Total Ca was determined by the method of Rothwell.[9] Ultrafiltration of the human milk was continued overnight. This was necessary because it was found that in order to obtain 3 cc. of ultrafiltrate from human milk 6 to 24 hours were required, whereas this amount was obtained with cow's milk in less than 3 hours. The explanation for this phenomenon is wanting. Skimming the human milk did not hasten ultrafiltration. The viscosity of this skimmed milk was found to be less than that of skimmed cow's milk. After removal of the casein with acid and rennin the whey of human milk still filtered more slowly than that of cow's milk. This experiment was not entirely satisfactory, however, since it was found impossible to obtain entirely clear whey from human milk by this means. That the prolonged ultrafiltration did not alter the composition of the ultrafiltrate was ascertained by a comparison of ultrafiltrates of the same sample of cow's milk obtained after 3 and after 24 hours.

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[6] Ballowitz, K., *Biochem. Z.*, 1932, **256**, 64.

[7] Marui, S., *Biochem. Z.*, 1927, **173**, 381.

[8] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1926, **66**, 375.

[9] Rothwell, C. S., *J. Biol. Chem.*, 1925, **65**, 129; 1927, **75**, 23.

TABLE I.

	Ca mg. per 100 cc.				Inorganic P mg. per 100 cc.			
	Milk		Ultrafiltrate		Milk		Ultrafiltrate	
Samples of milk*	Total	Adsorbable	Total	Adsorbable	Total	Adsorbable	Total	Adsorbable
Human A—Whole	25.2	14.0	17.9	7.1	8.4	5.1	5.1	2.2
" B "	23.4	11.6	14.4	5.3	7.4	5.4	2.5	1.1
" C "	—	—	14.8	5.5	3.6	—	2.9	1.8
" D "	34.8	16.8	21.8	14.1	7.2	3.8	6.1	3.1
" E "	24.6	10.0	19.4	7.2	5.1	3.6	3.8	2.9
" C—Skimmed	23.0	11.1	15.1	4.8	3.5	2.6	3.0	1.8
" D "	32.8	13.4	21.6	14.3	8.3	—	4.2	1.9
" E "	25.5	10.7	18.2	7.8	5.3	—	3.1	2.7
Cow's A—Raw	129.6	—	37.2	—	66.3	21.2	34.1	13.5
" B "	118.8	70.5	38.5	21.4	69.4	32.3	34.3	6.1
" C "	129.3	90.9	41.9	17.0	73.4	39.8	38.6	11.6
" D "	129.9	76.0	35.7	10.7	76.0	29.8	37.3	9.6
" B—Pasteurized	119.3	50.8	38.8	23.2	63.4	30.1	34.7	10.3
" E "	122.4	61.6	38.4	16.3	74.5	39.9	34.8	11.5
" F "	133.4	89.0	33.8	18.0	79.7	38.6	36.4	14.7
" G—Raw, irradiated	131.4	85.3	35.1	17.3	73.0	46.9	39.1	14.9
" H—Pasteurized, irradiated	131.9	99.3	34.6	18.1	76.0	41.3	36.6	14.7
" I " "	108.0	53.5	33.3	15.7	68.8	28.2	29.1	5.9
Averages		%	%	%		%	%	%
Human	27.2	12.5 (47)	17.9 (66)	8.3 (30)	6.1	4.1 (67)	3.8 (62)	2.2 (36)
Cow's, raw	126.9	79.1 (62)	38.3 (30)	16.3 (13)	71.3	30.9 (43)	36.1 (51)	10.2 (14)
" pasteurized	125.0	67.1 (54)	37.0 (30)	19.2 (15)	72.5	36.2 (50)	35.3 (49)	12.2 (17)
" irradiated	123.8	79.3 (64)	34.3 (28)	17.0 (14)	72.6	38.8 (54)	33.9 (47)	11.8 (16)

*The samples of human milk were all individual, and varied from 10 days to 5 months postpartum. Those of cow's milk were all composite.

It may be seen from the table that a partition somewhat similar to that of the Ca of serum may be made of the Ca in milk. The total may be divided into (1) a filtrable adsorbable fraction; (2) a filtrable remainder, probably containing the ionized fraction (column 3 minus column 4); (3) a non-filtrable, adsorbable fraction (column 2 minus column 4), and (4) a non-filtrable remainder, possibly bound to protein (column 1 minus column 3). In respect to the relative amounts of these fractions, human milk resembles serum, whether human or bovine, more closely than does cow's milk. In human serum 45% of the Ca is capable of adsorption by $BaSO_4$, 55% is ultrafiltrable, the filtrable adsorbable Ca-P complex constituting 35% of the total. These figures compare closely with the corresponding values for human milk: 47, 66, and 30%, respectively. *The filtrability of the Ca in human milk is more than twice as great as that in cow's milk, and it contains a much larger proportion of the filtrable adsorbable Ca fraction.* Thus it would seem that, for purposes of bone formation, the Ca in human milk is in a more available form than that in cow's milk. On the other hand, the absolute amount of the filtrable adsorbable fraction of Ca is small in human milk in comparison to that in cow's milk, so that even a two-thirds dilution of the latter would supply the infant with a greater amount of this particular form than undiluted human milk. It is, therefore, unlikely that these differences in composition are directly responsible for the greater efficacy of human milk in the prevention of rickets.

As in the case of serum, irradiation of cow's milk had no effect on the Ca partition. This held true also for pasteurization.

The partition of inorganic P in milk is different from that in serum. In the latter, the inorganic P normally is entirely ultrafiltrable and only 2 fractions can be separated by the use of the technic employed. In milk, on the other hand, only about one-half to one-third of the total P is found in the ultrafiltrate. Furthermore, not all of the adsorbable P is ultrafiltrable so that the P of milk can be separated into 4 fractions, corresponding to the 4 calcium fractions. Indeed, the relative concentration of the filtrable adsorbable P in the various samples of milk closely parallels that of the filtrable adsorbable Ca.

As with the Ca, the absolute amount of all fractions of P was greater in cow's than in human milk and likewise, the partition was not altered either by irradiation or by pasteurization.

6932

Effect of Luteinizing and Follicular Stimulating Fractions of Pituitary on the Thyroid.*

ROY GREEP. (Introduced by Frederick L. Hisaw.)

From the Department of Zoology, University of Wisconsin.

That the anterior lobe of the hypophysis exerts a definite action on the thyroid gland seems well established. Although considerable work has been done (Loeb,[1] Riddle,[2] Schockaert,[3] Collip[4]) it is yet a point of discussion as to which factor of the anterior lobe is responsible for this action. The preparation of a luteinizing and a follicular stimulating fraction in this laboratory (Fevold et al[5]) has made it possible to test the thyroid stimulating ability of 2 extracts which differ widely in their physiological action on the ovaries. The gonadotropic ability of the extracts was determined before their thyreotropic action was tested. The follicular stimulating fractions used produced from 300 to 800% increase in weight of immature rat ovaries with production of little or no luteinization, while the luteinizing fraction even in comparatively large doses, had little or no effect.

A series of 9 young male and female guinea pigs from 3 to 12 weeks old were given subcutaneous injections of the luteinizing fraction (0.5 to 3 gm. equivalent pituitary powder) twice daily over a period of 5 days. The thyroid glands and ovaries were weighed and prepared for histological study 12 hours after the last injection. A similar group of 5 guinea pigs were injected with the follicular stimulating fraction (0.5 to 2 gm. equivalent). In each of these groups litter mate controls were kept and autopsied at the same time as the experimental animals.

The luteinizing fraction produced an increase in the weight of the thyroids of 50 to 145%, average 103%, over those of litter

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* Aided in part by a grant from the National Research Council, Committee on Problems of Sex, administered by Frederick L. Hisaw.

[1] Loeb, Leo, *Endocrinol.*, 1932, **16**, 129.

[2] Riddle, O., Bates, R. W., and Dykshorn, S. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 794.

[3] Schockaert, J. A., *Am. J. Anat.*, 1932, **48**, 379.

[4] Anderson, E. M., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 680.

[5] Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Physiol.*, 1933, in press.

mate controls. At the time of autopsy these glands appeared swollen and highly vascular. Upon microscopic examination the acinar epithelium, which in the normal animal is cuboidal, was invariably found to be of a very tall columnar type. The follicles were irregular in outline and the follicular epithelium was thrown into numerous folds. The lumina of the acini, which in untreated animals are typically filled with solid colloid, were found to be empty. In some follicles the lumina had become obliterated by enormously elongated epithelial cells. The cytoplasm of these enlarged cells was filled with granular material and the nuclei were quite vesicular. On the whole, the thyroid picture was one of excessive secretory activity.

The follicular stimulating fraction produced no significant change in the weight of the thyroid. Histologically these glands were quite normal in every respect with the exception of 2 animals. In each of these the thyroid was somewhat atrophic. The acinar epithelium was flattened and the nuclei of the epithelial cells were likewise flattened and pycnotic.

It was found that neither castration nor sex altered the response of the thyroid to either of the gonadotropic substances. Also, similar experiments, in which young female rats were used, gave essentially the same results as those for guinea pigs.

Other preparations such as placental extracts, prolan from pregnancy urine, and corporin, produced no significant changes either in the weight or the histological appearance of the guinea pig thyroid.

The data presented here indicate that thyroid hyperplasia was produced either by the luteinizing principle or by another substance associated with it in the preparation.

6933

Hypophysectomy and Thyroidectomy of Snakes.*

WALTER H. SCHAEFER. (Introduced by Frederick L. Hisaw.)
From the Department of Zoology, University of Wisconsin.

The effects of hypophysectomy on mammals and amphibians have been extensively investigated, but apparently no work of this nature has been done on reptiles. The present study on garter snakes *(Thamnophis sirtalis* and *T. radix)* was made to determine whether or not hypophysectomy of reptiles produces the same results known for other vertebrates. Several male snakes were hypophysectomized to determine the histological changes of the endocrine organs, while others were given homeoplastic hypophyseal implants.

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* Aided in part by a grant from the Committee for Problems of Sex of the National Research Council, administered by Dr. Frederick L. Hisaw.

The testes of hypophysectomized animals became small and flaccid with degenerating spermatocytes in the atretic seminiferous tubules, while the size of the interstitial cells was less than normal. Subcutaneous implantation of a single hypophysis every other day for a period of 30 days, elicited germ cell production, enlarged the tubules, and increased the size of the interstitial cells. The adrenal glands after hypophysectomy were reduced to about two-thirds normal size. The cortical cells were atretic, and most of the nuclei had migrated to the periphery of the cell cluster. No retrogressive changes were noticed, however, in the cells of the medullary tissue. Hypophyseal implants, as given, did not restore the normal size of the gland, but the cells regained approximately their normal appearance and nuclear distribution.

The acinar epithelium of the thyroid gland, which is normally cuboidal, was, after hypophysectomy, reduced to a squamous type and these modifications were definitely although not entirely corrected by hypophyseal implants. Removal of the hypophysis also invariably caused shedding† of the skin which appeared to be due to thyroid disturbance. The first shedding occurred between 13 and 30 days after which the animals continued to shed at irregular intervals ranging from 22 to 70 days. One animal of this group shed 5 successive times, while normal animals kept under the same dietary and environmental conditions shed only once. Benedict,[1] in his studies on the basal metabolism of reptiles, found considerable deviation during the shedding period, and inferred that the shedding is not without influence upon metabolism. The present study suggests that the thyroid gland in some manner controls the shedding process. Two snakes which were thyroidectomized shed 15 days later, and entered shedding cycles similar to those of hypophysectomized snakes; one sloughing 5 and the other 6 times. That such shedding is due to the absence of thyroid activity is demonstrated by the fact that the feeding of 0.5 mg. of desiccated thyroid tissue for each gram of body weight to both hypophysectomized and thyroidectomized snakes prevented shedding. About 30 snakes were put under hibernating conditions for several months, and none of them shed its skin during that time. This indicates that the shedding is not induced by lowered metabolism alone, but probably is due to the absence of thyroid activity.

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† The word "shedding" as used in this report refers not only to the actual sloughing of the skin, but also to the first appearance of milky eyes which initiates the shedding process.

[1] Benedict, F. G., Carnegie Institution of Washington, Pub. No. 425, 1932, 163.

6934

Purification of Phage by Adsorption and Elution.

I. J. KLIGLER AND L. OLITZKI.

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

A precise study of the nature of viruses is rendered difficult by various extraneous substances. Although these substances do not appear to affect the action of the virus on its specific host, they modify the behavior of the virus in tests designed to ascertain its nature. Recently two methods have been reported for purifying viruses. One is based on ultrafiltration,[1] the other on adsorption and elution.[2]

The latter procedure is more easily carried out in the laboratory. However, it is difficult to standardize the technique and hence some authors have reported positive, others negative results. This paper deals with the standardization of the technique and the further purification of *B. coli* bacteriophage.

Media and Reaction: The character of the medium influences the adsorption, elution and amount of phage produced. In a synthetic medium the concentration of phage at the outset is low and elution is unsatisfactory. Adsorption is best in an acid and elution in an alkaline medium. The following protocol is typical:

Parallel tubes of broth and synthetic media were seeded at the same time with the same amounts of phage and culture, incubated 24 hours at 37°C., and filtered through a Seitz filter.

The phage titre in the broth was 10^9, in the synthetic media 10^5.

Method of Eluting. Adsorption is more complete at pH 5.5 than

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[1] Elfold, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[2] Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

TABLE I.

	Phage Concentration*		
	Supernatant fluid after adsorption	Resuspended kaoline sediment	Eluate**
Broth			
Adsorbed with kaoline at:			
pH 5.5	10^2	10^6	10^8
pH 7.6	10^3	10^9	10^7
Synthetic medium			
Adsorbed with kaoline at			
pH 5.5	0	10^3	10^2
pH 7.6	10^1	10^4	10^1

*The numbers equal phage plaques.
**See reference.

at pH 7.6 and the former adsorbate yields the best eluate. In the protein containing broth the phage is not so completely removed as in the synthetic medium. On the other hand it is apparently less firmly bound to the adsorbent and more readily removed by the eluting fluid.

The kaoline is also a variable factor. Each lot must be tested for optimal concentration. A 50% suspension in saline adjusted with phosphate solution to pH 5.5 and autoclaved served as the stock. Adsorption was carried out by adding equal volumes of kaoline suspension and phage, the mixture shaken thoroughly and left overnight in the ice box.

Elution was most effective with a N/100 solution of N/100 NH_4OH. Twice the original volume was added to the kaoline sediment after thorough centrifugation and removal of the supernatant fluid.

Purification of the Eluate. The eluate thus obtained had a phage content of 10^8, gave negative tests with Esbach and sulfosalycilic acid and a positive ninhydrin reaction. The total non-ammonia N was 9 to 11 mg. per 100 cc.

Further purification was effected by readsorbing 4 cc. eluate with 2 gm. kaoline (pH 5.5), and eluting again with 8 cc., N/100 NH_4OH. The second eluate had a phage content of 10^7, and the ninhydrin test was slightly positive.

This eluate was dialized under sterile conditions for 6 days. The phage content then was 10^6, protein and ninhydrin tests were negative and the N content was 1.4 mg. per 100 cc. These results indicate a phage suspension of a high degree of purity. Experiments are now under way to determine the behavior and antigenic character of this purified phage.

The above experiments indicate that it is possible to obtain a potent phage practically free of extraneous proteins or amino acids.

6935

Cause of Onset of Labor. An Hormonal Investigation.

J. THORNWELL WITHERSPOON. (Introduced by C. C. Bass.)
From the Department of Gynecology, School of Medicine, Tulane University.

With the increase in our knowledge of the rôle played by the pituitary and ovarian hormones, attention is being directed toward the possibility of a hormonal source as a fundamental cause which induces labor. Animal experiments suggest that there is a synergistic action between oestrin and pituitrin. If the isolated uterus is first bathed with oestrin, it becomes more sensitive to pituitrin as manifested by increase of contraction.[1-5] On this hypothesis, a hormonal theory as to the cause of the onset of labor may be summarized as follows: During early pregnancy oestrin is held in abeyance by the presence of the luteinizing factor in the ovary, placenta, and anterior hypophysis, and as maturity of gestation approaches release of this inhibiting action upon oestrin is observed. The uterine sensitivity to pituitrin, stimulated by the released oestrin, becomes more marked and continues until the threshold is reached, when labor is precipitated.

To test this theory clinically, a series of hormonal injections was undertaken on 45 pregnant negro women at term. The ovarian follicular hormone, in the form of theelin, was administered in various dosages, singularly and daily, to 10 pregnant women who were at term. Labor occurred within 36 hours in only 2 cases. Ovarian follicular fluid, aspirated at operation, was given to 2 women in a similar state; neither delivered in the allotted time. This same hormone in the form of amniotin or progynon was administered alone, or in combination with pituitrin or pitocin, the active uterine stimulating principle of pituitrin, in various dosages and at different

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[1] Bourne, A. W., and Burn, J. H., *Lancet*, 1928, **2**, 1020.

[2] Brouha, L., and Simmonnet, H., *Compt. Rend. Soc. de Biol.*, 1928, **94**, 759.

[3] Dixon, W. E., and Marshall, F. N. A., *J. Phys.*, 1924, **59**, 276.

[4] Jeffcoate, T. N. A., *J. Obs. and Gyn. Brit. Empire*, 1932, **39**, 67.

[5] Witherspoon, J. T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1063.

intervals, to 30 full term pregnant negro women. Seven delivered within 36 hours. Assuming that there might be an oxytocic factor present in the blood of parturient women, 25 cc. of parturient blood taken from women in hard labor, were injected into the buttocks of 3 women who were at term. One delivered within 36 hours—actually in 11 hours. In no case was there any maternal or fetal abnormality attributable to the injections. Table I .indicates the findings in 45 full term negro women in whom labor apparently was induced in 22.2% by hormonal injections.

TABLE I.

	Delivered Within 36 hours	Failed
Theelin, 1 to 4 cc. singularly and daily	2	8
" 1 cc., with pituitrin, m. iv every 4 hours	0	3
Aspirated ovarian follicular fluid, 1 to 4 cc.	0	2
Amniotin, 1 to 3 pessaries, bid.	3	7
" 1 pessary bid. with pitocin, m. iv	0	1
" 1 cc. (hypo), with pitocin, m. iv every 4 hrs.	1	0
" 1 cc. (hypo), with pituitrin, m. iv every 4 hrs.	1	3
" (oral), 5 cc. daily	0	3
" (oral), 5 cc. with pitocin, m. iv bid.	1	2
Progynon, 1 cc., with pitocin, m. iv every 4 hours	0	1
" 1 cc., with pituitrin, m. iv every 4 hours	1	3
Parturient blood, 25 cc. (hypo)	1	2
	10	35

In several instances the onset of labor followed the injections so closely that induction of labor could possibly have been considered the result of the therapy. Hormonal induction of labor will be of value clinically only when our knowledge concerning the regulation or mechanism of labor is on a firmer basis.

My thanks are extended to the following drug firms which supplied the hormonal preparations. Theelin 1 cc. equals 50 R.U.; Pituitrin, and Pitocin (Parke-Davis). Amniotin 1 pessary contains 75 R. U.; 1 cc. (Hypo) contains 50 R.U. 5 cc. Oral contains 2000 R.U. (E. R. Squibb and Sons). Progynon 1 cc. contains 50 R.U. (Schering and Company).

6936

Sex Stimulating Principle in Extracts of Beef Hypophyses Effective in Female Dogs.

B. O. BARNES* AND J. G. BUENO. (Introduced by A. J. Carlson.)

From the Department of Physiology, the University of Chicago.

During a study of Loeb's anterior pituitary extract of cattle glands on the basal metabolism of dogs, it was observed that the females displayed some of the signs of estrus. About 4 days after beginning the injections, the external genitalia became swollen, mucus secretion appeared, and by the fifth day most animals showed blood in the vulva. Some of these observations were made in the winter when natural estrus is unlikely to occur. To further test the possibility of coincident estrus, 2 females received a second series of injections within a short time and a similar sexual response again occurred. No response has been observed in 2 ovariectomized animals which suggests that the ovaries are stimulated by the extract. Apparently the action on the sex glands is independent of the thyroid stimulation since several thyroidectomized dogs have responded like the normals. Loeb[1] has investigated the effect of this extract on the ovaries of guinea pigs and rats, and has not found the same type of stimulation which one sees after inoculation of certain pituitary glands. The dosage employed here is not excessive since the equivalent of 0.5 gm. of dry pituitary powder is effective in dogs weighing 10-15 kg. When the extract was injected into a puppy 5 weeks old for a period of 12 days, no external response was visible. Lactation has occurred in several of the adult female dogs. The histological picture is now being studied.

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* National Research Fellow.

[1] Loeb, *Endocrinol.*, 1932, **16**, 129.

6937

Diabetogenic Principle of the Anterior Pituitary.

EVERETTE I. EVANS. (Introduced by A. J. Carlson.)

From the Physiological Laboratory, Division of Dairy-Cattle Breeding, Feeding and Management, Bureau of Dairy Industry, U. S. Dept. of Agriculture.

In this laboratory we have been engaged in the preparation of potent extracts of the anterior pituitary for isolation of the lactation principle. While studying the effects of these extracts on the normal dog, the development of a severe lipemia was noted to occur some 24 to 48 hours after the first injection. Further investigation revealed that these extracts contained the diabetogenic principle of the anterior pituitary.

The writer communicated his findings to Dr. A. J. Carlson March 26, 1933, for criticism. Further, Dr. B. O. Barnes reported to the Physiological Society, Cincinnati meeting, April 11, 1933, that an acid extract of the anterior lobe produced glycosuria in 3 normal dogs. At that time, neither Dr. Barnes nor myself were aware of Houssay's report of the same nature. Thus, it seems that this observation was made independently in 3 laboratories, although admittedly earlier by Houssay.[1]

The summary of the author's evidence that a diabetic syndrome of varying intensity can be established in normal dogs by the subcutaneous administration of alkaline extracts of the anterior lobe follows. To date 12 dogs have been studied.

Lipemia: Within 24 to 48 hours after the first injection, a marked lipemia developed in 6 of the 12 dogs. In general, this lipemia did not persist beyond the fifth day. It may be that this lipemia is caused by a sudden unloading of the fat stores of the body into the blood stream.

Polydipsia: Within 24 to 36 hours, polydipsia develops, all dogs drinking about 1900 cc. per day. It was distinctly observed that the polydipsia preceded the polyuria.

Polyuria: Polyuria is not marked until the fifth day, the urine output averaging then about 1500 to 2300 cc. In most cases, the specific gravity of the urine would indicate a diabetes insipidus (about 1.006); in 5 dogs the urine sp. gr. rose to between 1.035 and 1.045.

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[1] Houssay, B. A., Biassotti, A., and Rietti, C. T., *Revista de la Soc. Argent. de Biol.*, 1932, **8**, 469.

Glycosuria: In 7 dogs, glycosuria was evident on the fifth day (mild). In 3 dogs, the glycosuria was marked. The D/N ratio was not determined. The presence of sugar in the urine follows rather closely the blood sugar level and does not appear until the blood sugar is about 145 mg. %.

Hyperglycemia: The fasting blood sugar (Shaffer and Hartman) in these dogs was about 93-113 mg. % before injection; it rises 48 hours after the first injection to 145-425 mg. %. This high sugar level persists for about a week (injections being continued) and then some compensation takes place, the blood sugar level falling to a sub-normal level (70-90 mg. %). In some dogs this apparent hypoglycemia persists for at least a month after the injections have been stopped. There is a distinct vagatonia during the initial hyperglycemic period but after the injections have been discontinued the dogs exhibit a fairly marked polyphagia.

Glucose tolerance test (Killian): Before the injections the dogs gave normal glucose tolerance curves. After injections, the curves indicate diabetes, the first hour sample showing a rise to 250-280 mg. %. Even after the fasting blood sugar has returned to normal, the glucose tolerance curves are abnormal. No opportunity has yet been afforded to simultaneously determine the R.Q. values during a test period.

Acidosis: In 3 dogs specifically studied, the pH (quinhydrone electrode 20°C.) dropped from a normal of 7.55-7.60 to 7.35, 7.41 and 7.42. In 2 cases the pH drop was accompanied by a lowering of the alkali reserve from a normal of 55 vol. % CO_2 to 35.2 and 41 vol. % respectively. These dogs went into coma which was partly relieved by insulin. The alkaline reserve was not restored to normal until bicarbonate was administered.

Attempts have been made to separate the diabetogenic principle from the lactation principle, so far with no success. It is evident, however, that the diuretic action of these extracts is probably not due to the "foreign protein" injected. Injection of the globulin fractions of the extract in comparable doses produced no increase in urine output of normal dogs.

6938

Initiation of Copious Milk Secretion in Virgin Goats by Anterior Pituitary.

EVERETTE I. EVANS. (Introduced by A. J. Carlson.)

From the Physiological Laboratory, Division of Dairy-Cattle Breeding, Feeding and Management, Bureau of Dairy Industry, U. S. Dept. of Agriculture.

Almost all the experimental data on the lactation principle of the anterior pituitary have been obtained from studies involving the use of the rabbit, guinea pig, rat or dove as the test animal. These species do not allow any quantitative estimation of the milk yield. Grüter and Stricker[1] demonstrated that in cows in normal lactation, it was possible through one injection of a potent extract of the anterior lobe to increase the milk yield 1 to 1½ liters per day. Asdell[2] reports that it was possible to prevent, "for a time at any rate, the normal decline in milk yield with advancing lactation" in the goat, by injections of anterior lobe extract (Parke, Davis & Co.). Using this same extract, Asdell was unable to significantly initiate milk secretion in a young virgin goat, nor was he able to effect any increase in the milk yield of a mature goat whose lactation was nearly spent.

In this laboratory we have prepared our own alkaline extracts of the anterior pituitary. Copious lactation has been induced in 3 virgin goats and one dry goat. Figure 1, a and b, illustrates in a striking manner the effect of daily subcutaneous administration of 15 cc. alkaline extract of the anterior pituitary for 6 days. This goat on her first milking gave 4 pounds of milk. Further, it should be emphasized that all attempts to induce secretion of milk from her udder before the injections were started, failed. The same is true of the other 2 virgin goats and the dry goat. After 10 days, the injections were discontinued because it was evident that no further increase in yield could be effected. The daily milk yield was then 3.5 pounds. It was found, however, that subsequent administration of the extract 40 days later effected a second increase in milk flow to a daily yield of 4.8 to 5.8 pounds.

The milk yield for the other 2 virgin goats 20 days after all injections had been stopped was 1.8 and 2.8 pounds, respectively. At

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[1] Grüter, F., Proc. Second Int. Cong. Sex Research, Oliver & Boyd, London, 1932, 443.

[2] Asdell, S. A., *Am. J. Physiol.*, 1932, **100**, 137.

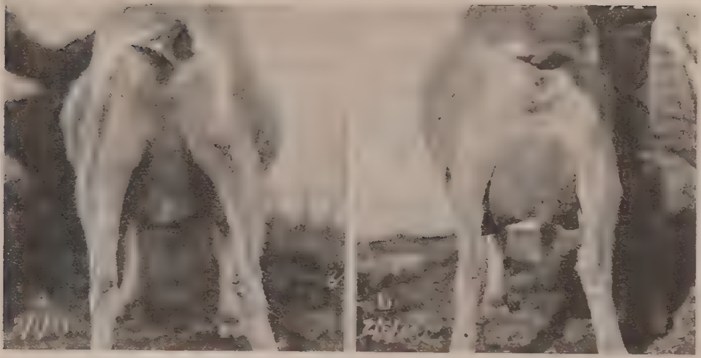

FIG. 1. a and b.
(a) 2/1/33 before injections; (b) 2/6/33 after six daily injections of 15 cc. anterior pituitary extract.

no time have any toxic symptoms been noted, even though as much as 40 cc. of the extract has been injected in one day to determine this effect. Further, these results have been checked on virgin cows and dogs; one cow is now giving 15.5 pounds daily.

In collaboration with Dr. I. P. Earle, we are making chemical examinations of the protein fractions of the first milks from these virgin goats and cows. Preliminary observations indicate that the first milk secreted under these experimental conditions (anterior lobe extracts) strongly resembles colostrum (globulin fractions, casein, percentage of total nitrogen, R.B.C.'s). These data present evidence that the secretion of colostrum may be controlled by the anterior lobe through its effect on the permeability of the blood vessels of the mammary gland.

6939

Saliva and Coagulation of Blood.

CARROLL J. BELLIS AND F. H. SCOTT.

From the Department of Physiology, University of Minnesota.

Following the observation that saliva markedly decreases the coagulation time of the blood,[1] studies were made to investigate the specificity of this substance. In our previous work we showed that human saliva collected from the mouth hastens the coagulation of dog, bovine, and human blood.

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[1] Bellis, C. J., Birnbaum, W., and Scott, F. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1107.

Dog saliva drawn from the cannulated Wharton's duct has the same accelerating effect, a fact also noted with bovine saliva. With a recalcification time of 6′ 15″, 1 cc. of bovine saliva added to 3 cc. of plasma gave a coagulation time of 4′ 40″, a reduction of 25%. The coagulant in saliva is, therefore, non-specific either in regard to its source or the source of the blood. This fact is strikingly manifest in the case of hemophilia. Hemophiliac blood, whose inability to clot is attributed both to a delay in the rupture of platelets to yield "tissue extract" and to a delay in the rate of conversion of prothrombin to thrombin, can be readily clotted by the addition of small amounts of saliva, either his own or that of normal individuals. In one individual, who had a clotting time of over 2 hours, the addition of 1 part saliva to 4 parts of his blood gave a solid clot in 3 minutes. Using the same volumes, but diluting the saliva 1:100 with 0.9% NaCl, the clotting time was 7 minutes with a normal retraction at the end of 45 minutes.

Similar to the effect of any injected animal secretion, a fall in blood pressure with an accompanying hyperpnoea was observed when 5 cc. or human saliva was injected intravenously into an etherized dog. The effect is quickly lost, and the pressure and res-

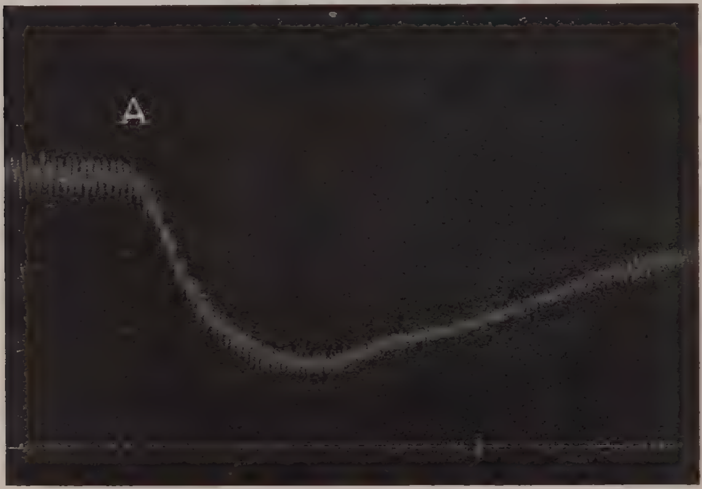

FIG. 1.

piration return to normal, whereupon the same effects may be elicited by repeating the injection. Fig. 1 represents the blood pressure effect on a dog, 5 cc. of human saliva having been injected at "A". The coagulation time of the animal's blood drawn after the injection shows a lengthening, rather than a shortening. This effect is probably similar to that observed after the injection of Witte's peptone or during the negative phase following injection of tissue extracts. The injection of large quantities of saliva subcutaneously in the dog (200 cc.) similarly delays coagulation.

TABLE I.

(a) Ratio of human peritoneal fluid to bovine blood and coagulation times.						
Normal	2:3	1:3	.6:3	.4:3	.2:3	.1:3
8′00″	4′40″	4′30″	4′10″	3′50″	2′00″	3′00″
(b) Ratio of human cerebro-spinal fluid to bovine blood and coagulation times.						
8′00″	—	4′45″	3′15″	3′00″	2′45″	3′30″

Fluid obtained by abdominal or intrathecal paracentesis shows the same coagulative properties as saliva. This is shown in Table I. It is evident that saliva is but one of several secretions which have the property of hastening coagulation.

6940

Concomitant Clearances of Creatinine, d-Xylose, Urea and Chlorides During Diuresis in Congestive Heart Failure.

GEORGE HERRMANN, E. H. SCHWAB, J. A. ALVAREZ and M. E. CATE.

From the Department of the Practice of Medicine, University of Texas School of Medicine, and the John Sealy Hospital, Galveston, Texas.

To get evidences of the mechanisms by which diuresis is accomplished in the edematous patient with heart disease, studies have been carried out on the concentration of various substances in the blood and in the augmented output of urine. Creatinine, or the total chromogenic clearance has been advocated by Rehberg[1] as a measure of the amount of glomerular filtration. The validity of this contention has been attacked by several investigators. Marshall[2] and Jolliffe, Shannon and Smith[3] have advocated the use of sugars and especially of d-xylose excretion and concentration as the index of the amount of glomerular filtration. Inasmuch as we[5] and others[1] noted great mobilization and increases in chloride and urea output during diuresis we[5] have included determinations of these substances in our studies. The urea clearance has been established by Van Slyke and his coworkers[4] as a measure of renal function. We have calculated in addition to this clearance, a similar but more arbitrary figure for chlorides, d-xylose and creatinine. The milligrams per cent of these substances in the urine and in the blood serum were determined simultaneously; dividing the former by the latter gives the degree of concentration or the index which is mul-

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[1] Rehberg, P. B., *Biochem. J.*, 1926, **20**, 447.

[2] Marshall, E. K., *Am. J. Physiol.*, 1930, **94**, 1.

[3] Jollife, N., Shannon, J. A., Smith, H. W., *Am. J. Physiol.*, 1932, **100**, 301.

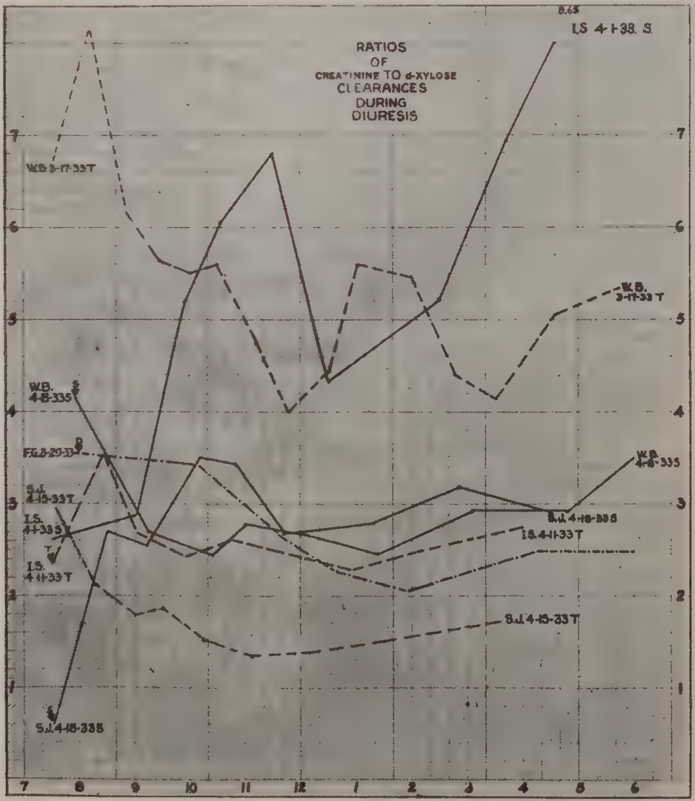

Fig. 1.

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[4] Van Slyke, D. D., and coworkers, *Medicine*, 1930, **9**, 1.

[5] Herrmann, G., Stone, C. T., Schwab, Edw. H., Bondurant, W. W., *J. Am. Med. Assn.*, **99**, 1647. *Trans. Assn. Am. Phys.*, 1932, **47**, 279.

tiplied by the volume of urine output in cubic centimeters per minute. For convenience we have called these final values "clearances", though they are not established as such for anything but urea. The figure represents directly the amount of blood flow through the kidneys necessary to yield the minute output. The normal values for urea have been established at 54 for urinary flow rates from 0.5 to 1.5 cc., and at 75 for rates above 2 cc. per minute; the respective

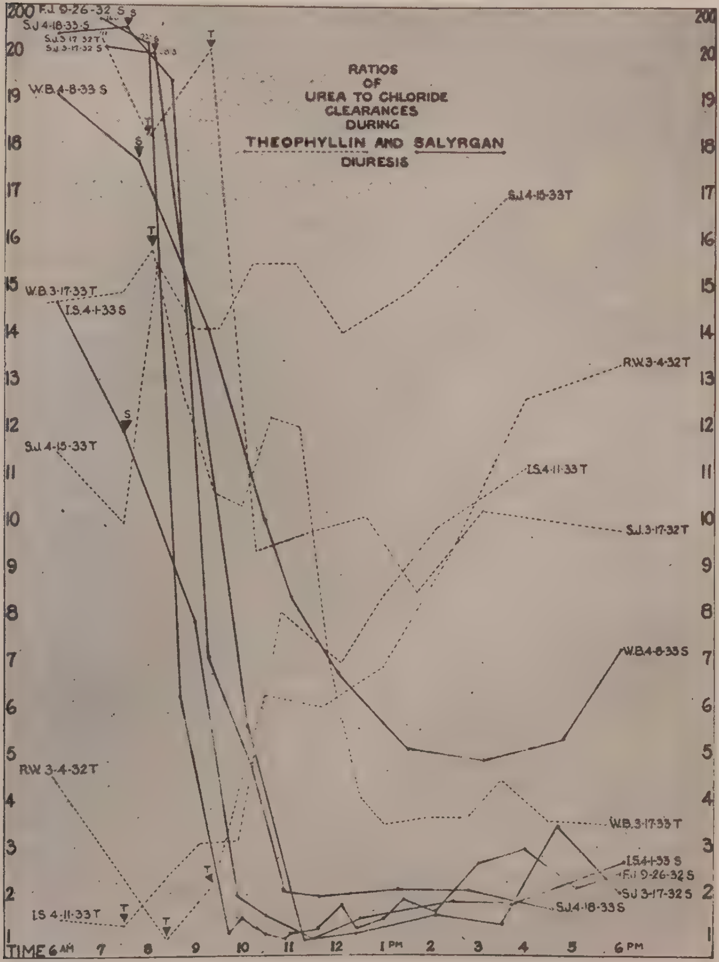

Fig. 2.

chloride rates are 0.5 to 7.5; the d-xylose rate about 90; and creatinine about 150. The ratios of the C (clearance) or F (filtration) of creatinine to d-xylose in normal men are about 1.75; those of urea to chlorides are about 100.

During our first series of 12 and our second series of 10 experiments in the diuresis produced in edematous cardiac patients we[5] noted striking differences in the clearance when the mercurial salyrgan was administered, in contrast to those obtained by giving the purine, thephyldine or by rapid digitalization with digalen. The clearance curves are of the same general types but d-xylose seems to act as a diuretic and increases the creatinine glomerular clearance which is evident especially during salyrgan diuresis. The ratios of the creatinine to d-xylose during the effective periods of the diuretic action are shown in Graph 1. The ratios are all far above the normal 1.75 level.

The urea to chloride ratios shown in Graph II demonstrate a striking difference in the effects of purine as contrasted to mercurial effects. Under the influence of salyrgan the ratios drop precipitately during diuresis, due to the decreased urea clearance and the tremendous rise in chloride output. In the diuresis of thephyldine and digalen there is a rising Ur/C (urea sodium chloride) ratio, the result of a conspicuous rise in urea clearance and a disproportionately small rise in chloride excretion. This very sharp contrast in ratios is evidence of a definite difference in the modes of action of the different types of diuretics. The findings support the view that we have subscribed to on the basis of our clinical experimental work[5] and that of Schmitz[6] on dogs, namely, that the mercurials act by decreasing tubular reabsorption primarily while the purines and digitalis increase glomerular blood flow and filtration primarily. The reabsorption of chlorides as threshold substances conserved by the body is apparently sharply interfered with as a result of the depressing action of salyrgan on the membranes or the cells of the tubular epithelium.[5, 6] Urea, on the other hand, as a non-threshold substance is normally not absorbed and only small amounts normally diffuse through the tubular membrane and so the reabsorption defect does not affect it. Augmented glomerular filtration incident to theophyllin and digitalization result in greater urea clearances and relatively slight sodium chloride outputs. Such findings are, therefore, in accord with previous experiences.

The other ratios of the filtration or clearance of creatinine and of d-xylose to the other substances have been calculated and have been found to show some suggestively consistent or characteristic relationships in the salyrgan as contrasted to the theophyldine or digalen type of diuresis.

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[6] Schmitz, H. L., *J. Clin. Invest.*, 1932, **12**, 1075.

6941

Threshold Values of Ultra-Violet Radiations.*

GEORGE H. MAUGHAN.

From the Department of Physiology, Cornell University.

The question of glazing the windows of modern homes and hospitals so that winter sunshine with its low content of ultra-violet may enter, and the newer problem of so-called "dual purpose" lighting of houses has raised the question of threshold values.

When one selects ultra-violet sources of greatly reduced intensity and thus reduces sufficiently the size of the unit of ultra-violet irradiation it will be found that the amount necessary for the first detectable effects is much below the amount necessary for complete prevention of rickets. This threshold amount is between one-fourth and one-sixth that needed for complete prevention. Rays from very low power sources, applied in the most advantageous way, can be made quite efficient and practical. The difficulty is that the distance factor and the covering of the animal, when varied, make such enormous differences that results in the hands of different investigators may seem entirely contradictory.

Two series of tests have been directed specifically toward the problem of threshold values. In one, completed in June, 1930, there were 8 groups of chickens containing 17 birds each and 6 pens of rats with 4 rats in each. The lamps were 60 watt Mazda CX bulbs of varying intensities of ultra-violet, except in one control group where a mercury arc was used. The intensity of ultra-violet from the tungsten filament of these lamps can be varied by differences in size and length of the filament. This alters the temperature, and the higher the temperature the greater the amount of ultra-violet. The irradiation in some of the groups was also increased by increasing the time of exposure. A second set of experiments recently completed made use of the quartz mercury arc for a control

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* Aided by a grant from the Heckscher Research Foundation.

group, a Model E-1 GE Sunlamp and three 115 watt CX bulbs, one 60 watt, one 300 watt and a third of 500 watt size.

It was found by comparison that the ordinary quartz mercury arc without reflectance is about 2,000 times as strong a source of ultra-violet as the 60 watt CX lamp. However, the latter with a reflector, the rays falling perpendicularly upon the legs of chickens, almost cured rickets in 28 daily exposures of 20 minutes each, the distance being 4½ inches from floor to lamp.

One hour daily exposure to this CX lamp without reflectance at a distance of 13 inches and with pens 26 inches in diameter give the first signs of prevention of rickets in animals allowed to walk about in the pen. This seems to be the threshold value for the prevention of rickets in chickens. Two hours' exposure to the same lamp gave approximately 50% the irradiation necessary for complete prevention.

Ten daily exposures of 30 minutes each, to a 60 watt CX bulb gave the first signs of recovery in the cure of rachitic rats. The lamp had no reflectance and was placed 4⅜ inches between the bulb and the floor of the cage. This we consider the threshold value for cure of rickets in rats. Radiations from a 300 watt Mazda CX lamp under the same conditions except that the distance was 8 inches instead of 4⅜ produced definite healing in all the rats, but not with maximum rapidity.

A 500 watt CX lamp at a distance of 11⅜ inches and 50 minutes exposure gave a rate of recovery only slightly less than maximum.

6942

Unexpected Differences in Distribution of Blood Groups among American Indians.

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The distribution of the blood groups among the American Indians has been a matter of considerable interest to both anthropologists and serologists, because of the unusually high incidence of group O among them, as reported by several investigators. On the basis of these findings it has been suggested by Snyder and others that the human family was at one time a pure group O species and that the American Indian became separated from the rest of mankind before A and B agglutinogens developed in the cells.

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* I wish to acknowledge my gratitude to Dr. H. F. Schrader, Senior Physician, Blackfeet Agency, Browning, Montana, for his assistance in obtaining blood specimens for this investigation.

The writer had the opportunity of examining specimens of blood from the Blackfeet and Blood tribes of American Indians to determine whether the same high occurrence of group O exists among them.

Brief Tribal History.[†] In the early days the "Blackfeet", which included the "Piegans" and "Bloods" of Canada, ranged from the Rocky Mountains east to the Sioux territory, south into what is now Wyoming, and north into Canada. The agency records show admixtures with other tribes such as Canadian Cree, American Cree, Chippewa, Cherokee, Snake, Shoshone, Sioux, Gros Ventre, Flathead, Kootanai, and Alaskan, as well as others. In the early days captive women and children were adopted by the tribe and later members of other tribes came and settled with the Blackfeet. The first white men to come into contact with the Blackfeet were probably the first trappers and traders that came up the Missouri River. These Indians made Fort Benton and other forts on the Missouri their trading centers. In classifying them as to their degree of Indian blood, only where there is white or Negro admixture are they shown on the records as mixed.

Samples of blood were collected from 350 Blackfeet Indians, 115 of which were full-bloods. Contrary to the findings of the other workers among various other tribes, these Indians (putative full bloods) proved to be only 23.5% group O, and 76.5% group A. A similar distribution was observed among another branch of the Blackfeet, namely the Blood tribe of Alberta, Canada, which gave 83.3% group A.

TABLE I.
Percentage Distribution of Blood Groups Among the Blackfeet Tribe of Indians.

Degree of Blood	No. Examined	Distribution							
		O		A		B		AB	
		No.	%	No.	%	No.	%	No.	%
Putative full bloods	115	27	23.5	88	76.5	0	0	0	0
Known mixed bloods	235	107	45.5	119	50.6	5	2.1	4	1.8

[†] The historical information concerning the Blackfeet Indians was supplied by Superintendent Forrest R. Stone of the Blackfeet Agency to whom we acknowledge our thanks.

That these unexpected findings are not due to some error is evident from the fact that distribution of blood groups among 104 Indians belonging to a number of tribes other than Blackfeet or Blood and secured by the use of the same typing sera was found to correspond with that reported by other investigators—that is 83.6% group O; 11.5% group A; 2% group B and 2.9% group A.B.

It does not seem unlikely that the Blackfeet and Blood tribes were at one time a pure group A people and that racial crossing and admixtures with other tribes has modified the blood group distribution among them.

These results suggest the necessity for reconsidering the origin of the American Indian.

6943

Vitamin G Potency of Purified Liver Preparations.

ERWIN BRAND, RANDOLPH WEST AND CHARLES J. STUCKY.

From the Department of Chemistry, New York State Psychiatric Institute and Hospital, and the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

Commercial liver extracts which have been used extensively in the treatment of pernicious anemia are known to be rich in vitamin G (B_2, anti-pellagric factor).[1] It was thought of interest, therefore, to determine whether vitamin G is present in a purified preparation of the P.A. factor.

Purification of Lilly liver extract 343 was carried out by methods described[2] by one of us (W.), the material used being a regenerated amorphous barium derivative precipitated from alcohol, 200-250 mg. of which gave a maximal reticulocyte response when injected into a pernicious anemia patient. Three preparations (A, B and C) of this material were used, about 1 gm. each was obtained from 400 gm. of the Lilly Extract.

Vitamin G deficiency was induced in young rats (21 days old) of the Wistar strain according to the technique previously described.[3]

A total of 55 animals were employed for the experiments. The depletion period usually lasted 3 to 4 weeks. The liver preparations were then administered as supplements to the basal G-deficient ration. The following groups of rats were employed: (1) animals which received various quantities of the liver preparations; (2) negative controls which received the basal G-deficient diet only; (3) positive controls which received the basal ration supplemented with

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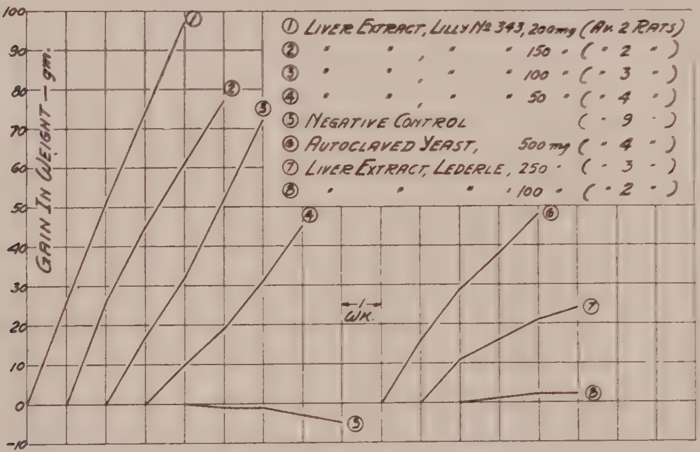
[1] Guha, B. C., *Biochem. J.*, 1931, **25**, 945.

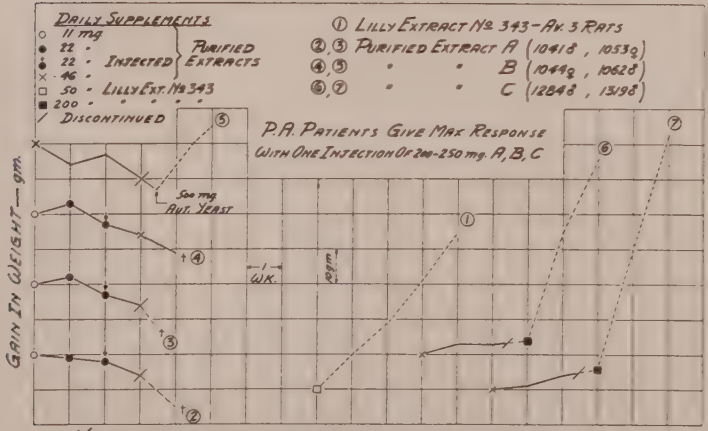
[2] West, R., and Howe, M., *J. Clin. Inv.*, 1930, **9**, 1.

[3] Stucky, C. J., and Brand, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1404.



VITAMIN G POTENCY OF LIVER EXTRACTS.

FIG. 1.



VITAMIN G POTENCY OF PURIFIED LIVER EXTRACTS

FIG. 2.

500 mg. of autoclaved yeast* daily. The experimental period lasted 3 to 5 weeks, the purified P.A. factor being administered orally and parenterally.

Typical data are presented in the charts. It may be seen that while 50 mg. daily of the crude liver extract gave adequate growth, 46 mg. of the purified P.A. material orally failed to support growth, nor did parenteral administration give better results. The addition of autoclaved yeast or crude liver extract was followed by immediate weight gain. Another liver extract (Lederle) presumably adequate in the antianemic factor, contained less vitamin G.

In addition to the data depicted in the graphs, assays for the vitamin G factor were performed with various fractions obtained during the preparation of the purified P.A. factor. The results were negative, which indicated that the vitamin G potency was lost during the initial stages of the purification.

It is hoped that the possible relationship between vitamin G and antianemic material from liver may be clarified by the reported preparation of nitrogen-containing fluorescent, pigment-like substances with a high degree of vitamin G activity.[4]

Summary. Purified liver extracts (P.A. factor) prepared from the Lilly extract No. 343 and of known potency in pernicious anemia were tested on vitamin G deficient rats. The extracts failed to induce growth in such animals. These experiments indicate that a purified preparation of the P.A. factor is lacking in vitamin G as tested by the growth method.

6944

Properties of Heated Chick Embryo Extracts When Activated.

JOSEPH T. KING. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

The writer has found that addition of one volume rabbit plasma to 60-100 volumes chick embryo extract greatly increases the coagulative power of the extract[1]; that the increase in power is due to a body which reacts like thrombin in regard to the calcium ion[2]; that the plasma factor involved is prothrombin[3]; that the extract factor has the heat stability of cephalin and that the heated extract is as susceptible to activation as the unheated.[4] (Degree of activation calculated according to the method of Mills.[5])

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* The yeast was obtained through the courtesy of Mr. Leo Wallerstein of the Wallerstein Laboratories, New York, N. Y.

[4] Kuhn, R., György, P., and Wagner-Jauregg, T., *Ber. d. chem. Gesell.*, 1933, **66**, 317.

[1] King, Joseph T., *Arch. f. Exp. Zellforsch.*, 1931, **10**, 467.

Further study has brought out the fact that the time at which activation occurs is closely correlated with the clotting power of the extract being activated (as measured by its power to shorten the coagulation time of a recalcified citrate rabbit plasma). There appears, however, to be no such correlation between the coagulative power of such an extract and the degree of activation.

Determinations of coagulative power and activation were done as described above.[4] In that paper it was reported that there was a marked decrease in the coagulative power of chick extract as heating was done at intervals of 10°C. and that the decrease was especially marked below 60°. Above this point the power is relatively stable at a much lower level.

With the decrease in coagulative power of the extract there is a lengthening of the latent period of activation. In a typical experiment 0.05 cc. fresh unheated extract shortened the coagulation time of a recalcified citrate plasma from 15 min. to 1 min. 30 sec.; activation required 4 min. 35 sec. After heating until the coagulation time increased from 1 min. 30 sec. to 7 min. the activation required 37 min.

The degree of activation is somewhat variable but there is not sufficient decrease to permit the conclusion that it is the thermolabile constituent of the extract that is responsible for the extent of activation. The data point to this constituent as determining the time at which activation occurs while the relatively heat stable constituent determines the extent.

The preliminary studies suggest that the thermolabile factor involved is the "tissue fibrinogen" of Mills. It probably does not directly activate prothrombin but brings about clotting of the activating plasma freeing the prothrombin.

When tested on a recalcified citrate plasma the increase in clotting power is found to take place during the time the fibrin is forming. Testing on a citrate plasma without recalcifying one finds that the ability to cause clotting appears at this same time. The control 0.05 cc. of either heated or fresh unactivated extract never causes clotting.

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[2] King, Joseph T., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1112.

[3] King, Joseph T., in press.

[4] King, Joseph T., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 868.

[5] Mills, C. A., and Matthews, Stewart, *Am. J. Physiol.*, 1922, **60**, 193.

6945

Influence of Oxygen on Survival of Tissue Suspensions.

JOSEPH T. KING AND LYDIA LUX. (Introduced by F. H. Scott.)

From the Department of Physiology, University of Minnesota.

Living cells are necessary for the growth of filterable virus. In certain instances cultures have been incubated with free access to oxygen. Li and Rivers[1] point out that a gradual simplification of the medium has taken place recently.[2, 3, 4] They also emphasize the advantages of chick embryo tissue in saline over the more complex media employed earlier. Flexner and Noguchi[5] and Long, Olitsky and Rhodes[6] on the virus of poliomyelitis are typical of work done on cultures under oil. Dochez, Mills and Kneeland[7] and Powell and Clowes[8] used chick embryo tissue under vaseline seal.

In this type of culture apparently intact cells are seen after incubation. Muckenfuss and Rivers[9] and Muckenfuss[10] found that the addition of dead cells to the virus improved survival. Eagles and McClean[11] emphasized the unsatisfactory state of our knowledge regarding the rôle of tissue which had access to oxygen.

We attempted to define more accurately the condition of the cells supposed to be living in such preparations. Saline suspensions of chick embryo were studied since they are coming into general use.

Ten-day chick embryos were shaken with freshly broken pyrex glass in 10 cc. of Tyrode as described earlier.[12] Three cc. portions of the suspension were incubated in pyrex test tubes under sterile oil at 37.5° for the intervals stated. Control consisted of 2 cc. of the same suspension in a Carrel D5 flask. Immediately after preparation and at intervals of one hour 0.2 cc. portions were removed after mixing and planted in Carrel D3 flasks in 1 part heparinized rabbit plasma and 2 parts tissue extract, a medium well known to promote rapid growth. Some of the same suspension was incorporated into a tissue culture clot, fixed, sectioned and stained for microscopic examination. Only the results on planted suspensions are reported in this preliminary note.

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[1] Li, C. P., and Rivers, *J. Exp. Med.*, 1930, **52**, 465.

[2] Carrel, A., and Rivers, T. M., *Compt. rend. Soc. Biol.*, 1927, **96**, 848.

[3] Maitland, H. B., and Maitland, M. C., *Lancet*, 1928, **2**, 596.

[4] Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 149.

[5] Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, **18**, 461.

[6] Long, P. H., Olitsky, P. K., and Rhodes, C. P., *J. Exp. Med.*, 1930, **52**, 361.

[7] Dochez, A. R., Mills, K. C., and Kneeland, Y., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 513.

[8] Powell, H. M., and Clowes, G. H. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 332.

[9] Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 149.

[10] Muckenfuss, R. S., *J. Exp. Med.*, 1931, **53**, 377.

[11] Eagles, G. H., and McClean, *Brit. J. Exp. Path.*, 1929, **10**, 35.

In general it has been found that incubation under oil as described causes serious damage to the tissues as expressed by an increase in the latent period of growth and a decrease in the vigor of the growth. Finally there is complete failure of growth.

In the control cultures at the beginning the latent period may be as short as 1½ hours and after growing for 48 hours the growth is dense. In the cultures from under oil the increase in latent period may be detected by the 4th or 5th hour and by the 12th hour there is serious impairment of growth. The impression gained is that failure of growth occurs not later than 16-20 hours. Control cultures from the Carrel flask at 24 hours showed practically no difference as compared with that planted at the beginning.

It is realized that the ability to grow is not the only criterion that may be utilized in determining the condition of cells in these preparations. Correlation of the histological and hydrogen ion changes is being made.

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[12] King, Joseph T., *Arch. f. Exp. Zellforsch.*, 1930, **10**, 341.

6946

Bacterial Allergy to Secondary Organisms in Pulmonary Tuberculosis.

F. M. HUNTOON.

*From the Research Laboratories, National Variety Artists Lodge, Saranac Lake, N. Y.**

Although it has long been known that secondary organisms occur almost constantly in the lung sputum of cases of pulmonary tuberculosis, particularly in the advanced cases with cavity formation, the possible clinical significance of the presence of such organisms has received little attention; and the consensus of clinical opinion has been that they are of little or no importance.

As far as the author is aware, no attempt has previously been made to determine such significance from the standpoint of acquired allergy. Experience over a period of 2 years has shown: (1) Pulmonary tuberculosis patients are often allergic (skin sensitive) to such secondary organisms. (2) Such allergy is sometimes accompanied by cough and expectoration not referable to the tuberculous condition. (3) Desensitization of such patients is often followed by improvement in cough and expectoration with coincident improvement in sedimentation rate, blood count and clinical condition. (4) A group of organisms often implicated in this connection is the Neisseria gram negative cocci, previously overlooked or ignored in this connection.

Organisms of the Neisseria group in the order of frequency as isolated in our studies are: *Neisseria perflava*—a large majority; *Neisseria sicca; Neisseria flava; Neisseria catarrhalis* (one instance, patient was not sensitive); *Neisseria subflava* (this has not yet been isolated in any of our cases). Two cases have been found with a double infection with the perflava and the flava. In each instance the case was sensitive to both types.

Another gram negative coccus has been isolated which ferments only dextrose, but by other cultural reactions does not belong in this group. Three cases with this organism have been found. This apparently undescribed organism will be reported elsewhere.

The determination of allergic patients naturally falls into: (1)

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*This investigation was initiated at the White Haven Sanatorium, White Haven, Pa., continued at the Trudeau Research Laboratories, Trudeau, New York, and at the National Variety Artists Lodge, Saranac Lake, N. Y.

Isolation of the secondary bacteria. (2) Preparation of a proper antigen. (3) Determination of the sensitiveness of the patient.

Isolation of secondary bacteria: A small portion of the mucopurulent lung sputum is washed through many changes of sterile salt solution, to remove contaminating mouth bacteria, and is then stroked over 2 consecutive plates of dextrose serum agar. A drop of sterile blood is often mixed with the sputum at the time of stroking. Dextrose is added about 0.01%, as it increases growth and facilitates the recognition of the gram negative cocci. At the end of 24 and 48 hours, the plates are examined, suspicious colonies fished to plain hormone agar slants, or if too crowded to fish pure, are fished and replated. The gram negative cocci are recognized by the glistening opalescent appearance of the colonies which may have a lemon yellow color. The usual flora is rather restricted. Nonhemolytic streptococci are almost always present. *Staphylococcus albus* and the *Micrococcus tetragenus* are common. In a majority of the cases studied, gram negative cocci have been present. We have never found a case sensitive to the *M. tetragenus* and seldom to the *Staphylococcus* or *Streptococcus*, although the latter is worth a trial in the failure of other organisms.

Preparation of the antigen: An emulsion of approximately one to two thousand million organisms per cc. is prepared in physiological salt solution. Some of these strains do not make good emulsions but break up perfectly after heating. The emulsions are transferred to 5 cc. ampul vials, the rubber stopper is tied in and the vial dropped in boiling water for 35 minutes. The use of such boiled antigen may seem theoretically inadvisable, but experience has shown that such boiled emulsions retain an antigenic factor which causes skin reactions in the sensitive patient and are therapeutically useful. This will be reported later.

Determination of sensitiveness: The patient is injected intradermally with 0.05 cc. of the heated antigen and observed during a course of 15 minutes and again at the end of 24 hours. Two reactions are possible, an immediate reaction in the first 15 minutes, characterized by a wheal surrounded by an area of erythema, a reaction similar to that elicited by horse serum or egg white in the sensitive patient. This reaction fades in the course of a few hours and may or may not be followed by a secondary reaction, resembling a tuberculin reaction, with a swollen red area from 2 to 5 cm. in diameter. At times, one reaction occurs without the other, but either seems to be an indication of general sensitiveness.

Assuming that the mucous membranes of the bronchial system are sensitive, it seems logical to assume that the presence of such organisms or their products in the lung sputum acts as an irritative factor, causing increased cough and excessive expectoration and that a proper desensitization would in a degree relieve such symptoms. This hypothesis has been borne out by clinical experience.

Desensitization: Owing to the clinical condition of most of the patients treated, it is important to avoid general reactions, and small doses frequently repeated are employed. The first subcutaneous dose is the same as the skin test dose, 0.05 cc. Injections are made every second day, if no reaction persists, raising the dose gradually to a maximum of 0.5 cc. to 0.75 cc. The interval is then increased to 4 days and the dosage held at this point. Treatment to continue 6 weeks to 2 months. If the treatment is to prove beneficial, improvement is usually apparent within 2 weeks. Disappointments are naturally encountered, but a sufficient number of patients are definitely benefited under such treatment to justify the labor and care involved.

Four such cases of many are cited in detail:

Case E. C. Chronic case with a progressive lesion and cavity, Gaffky IV. Very bad cough. Surgical interference contemplated. Sputum examination. *Streptococcus non hemolyticus* isolated. Vaccine prepared. Skin test. Moderate rather doubtful reaction. Vaccination over period 2 weeks. Patient worse. Vaccination stopped. Second sputum examination. *Neisseria perflava* isolated. Vaccine prepared. Skin test—immediate and marked reaction. Vaccination, followed by immediate and progressive improvement. Continued for 2 months. Cough and expectoration markedly diminished. Gaffky count decreased. Blood picture improved and a marked improvement in clinical condition. Skin reaction markedly diminished.

Case E. J. Very chronic case, cavities in both lungs, very ill. No improvement on 6 months' bed rest. Very annoying and racking cough. Sputum examination. *Neisseria sicca* isolated. Vaccine prepared. Skin test—marked reaction. Vaccination over a 3 months' period. Cough markedly diminished. Blood picture very much improved. Sedimentation rate steadily decreased. General condition improved. Skin reaction negative at this time.

Case G. D. Relapse in chronic tuberculosis. Both lungs affected. Cavitation on one side. Terrific racking cough causing vomiting. Sputum examination. *Neisseria perflava* isolated. Skin reaction to

N. perflava marked, both immediate and delayed. Vaccination over 3 months' period. After first week, cough progressively improved. Sputum raised with ease, amount lessened. This was followed by progressive improvement in clinical condition, in blood count and blood sedimentation. Gaffky count became negative. X-ray plates showed clearing. Second skin test at end of 4 months shows marked diminution of immediate reaction and disappearance of secondary reaction.

TABLE I.

No.	Case	Diagnosis All Pulmonary Cases	Organisms isolated and employed for vaccination	Results
1.	E.C.	Chronic tuberculosis cavitation	*N. perflava*	Markedly improved
2.	E.J.	Very chronic tuberculosis, cavitation both lungs	*N. sicca*	" "
3	G.D.	Relapse in chronic tuberculosis. Very severe cough	*N. perflava*	Immediate and progressive improvement
4.	M.S.	Relapse in chronic tuberculosis. Severe cough. Tenacious sputum	*N. sicca*	Immediate and progressive improvement
5.	F.D.	Chronic tuberculosis cavitation. Spread	*N. flava*	Marked improvement
6.	K.V.	Chronic tuberculosis	*N. perflava*	Improvement
7.	N.Q.	" "	" "	Slight but distinct improvement
8.	M.J.	" "	Unknown gram. Negative coccus	Improvement
9.	T.G.	" " Chronic myocarditis	*N. perflava*	"
10.	L.G.	Chronic tuberculosis	" "	Marked progressive improvement
11.	K.P.	Chronic tuberculosis. Violent cough, persistent blood streaking	Pneumococcus IV *N. perflava*	Marked improvement in cough. Bleeding stopped
12.	P.H.	Asthmatic symptoms following arrested tuberculosis	" "	Improvement
13.	P.M.	Asthma of long standing. Tuberculosis arrested	" "	"
14.	L.H.	Excessive expectoration. Asthmatic symptoms. Arrested tuberculosis	Unknown gram Negative coccus	No improvement
15.	L.G.	Thoracoplasty case. Persistent cough and expectoration	Unknown gram Negative coccus	" "
16.	D.A.	Purulent bronchitis	*Streptococcus non-hemolyticus*	Immediate improvement
17.	T.C.	Chronic bronchitis complicating a chronic sinus infection	*Streptococcus non-hemolyticus* *N. perflava*	Marked improvement

Case M. S. Relapse in chronic tuberculosis. Terrific cough due to extremely tenacious quality of sputum. *Neisseria sicca* isolated from sputum. Marked skin reaction. Immediate improvement in cough on vaccination due to change in character of sputum. Became more watery and less tenacious. Vaccination over 3 months. Progressive improvement and return to work. Skin test at end of this period. Reaction very slight.

In Table I are given briefly the findings and results in 17 cases, of which 11 were acutely ill of pulmonary tuberculosis at the time of treatment.

It may be argued that improvement in the cases cited is due not to desensitization but to an immunizing process, *i. e.*, the production of antibodies.

Against this view are the noted facts that the skin reaction diminishes in intensity, that the local reaction about the point of injection noted with the first few doses, quickly disappears, that if the interval between doses is too prolonged (from 5 to 7 days) the cough is apt to return, to be relieved promptly on the subsequent injection, and that overdosage in the first stages of the treatment may be followed by an exacerbation of symptoms, all point to the validity of the sensitization hypothesis and that a desensitization is the determining factor in the control of symptoms. Furthermore, animal experiments with the boiled antigens have shown that this antigen does not stimulate the production of antibodies except in a minor degree. This hypothesis of sensitization is no different from that assumed in cases of asthma, suffering from bacterial allergy.

The possibility of a non-specific effect must also be considered. In the first case cited, "E. C.", the patient was first vaccinated with a streptococcus, to which she was only mildly sensitive and with no benefit. A subsequent vaccination with an organism to which she was markedly sensitive was followed by immediate improvement. Several similar instances have occurred indicating a considerable degree at least of specificity.

Conclusions. Sufficient experience has been gained to demonstrate that: (1) Tuberculous patients are frequently allergic to secondary organisms present in the lung sputum. (2) Such organisms are often of the Neisseria group. (3) Desensitization of the allergic patient may be followed by marked improvement of the symptoms, cough and expectoration. This method in certain cases is a valuable adjuvant treatment in cases of pulmonary tuberculosis.

6947

Origin of Regenerating Brain in the Earthworm.

BENJAMIN KROPP. (Introduced by A. J. Goldforb.)

From the Zoological Laboratory, Harvard University.

Hescheler[1] described in detail the histogenetic processes following decapitation of the earthworm. He stated that the cerebral ganglia are formed from cells of the body wall ectoderm which have migrated posteriorly and medially. Nuzum and Rand[2] showed that this method of cerebral ganglia regeneration is by no means constant in *Allolobophora foetida* and that under certain conditions the epithelium of the dorsal pharynx wall may also contribute to brain formation. The latter had dissected only the cerebral ganglia from the head leaving the pharynx wall uninjured, whereas Hescheler's description was based on removal of 5 head segments.

In the course of a study of regenerating earthworm heads from another viewpoint, evidence confirmatory of Nuzum and Rand's findings was uncovered. In the present work 5 head segments were removed from *Helodrilus caliginosus* and in some cases 10 segments of ventral nerve cord in addition. In most specimens cere-

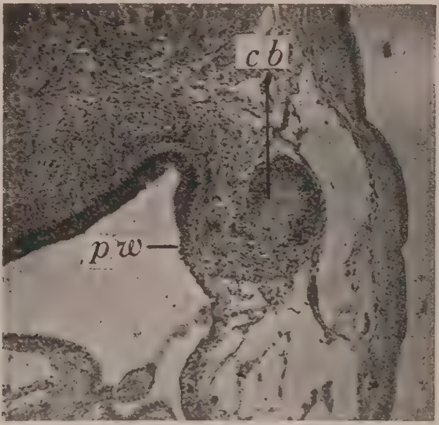

FIG. 1.
Animal IC 104. Formation of cerebral ganglia from dorsal pharynx wall, 26 days regeneration. Dorsal margin of animal above, anterior to right. pw = dorsal pharynx wall, cb = regenerating cerebral ganglia. × 64.

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[1] Hescheler, K., *Jenaische Z. f. wiss. Zool.*, 1898, **31**, 521.

[2] Nuzum, M. F., and Rand, H. W., *Biol. Bull.*, 1924, **47**, 213.

bral ganglia regeneration followed closely the course described by Hescheler. In several cases, however, there was an obvious contribution from both body wall ectoderm and dorsal pharynx wall to the regenerating brain. In one striking instance (Fig. 1) only cells from the epithelium of the dorsal pharynx wall were passing to the regenerating ganglia and no evidence of movement of cells from the dorsal ectoderm was anywhere apparent. In this specimen there was evident a stream of epithelial cells passing dorsally and laterally, in a spiral manner, from the dorsal pharynx wall epithelium towards the position occupied by the new brain. The muscle and connective tissue layers of the pharynx wall have not regenerated and a basement membrane is lacking in this region. There are numerous mitoses in the cells of the dorsal pharynx epithelium immediately posterior to the regenerating cerebral ganglia and within the brain itself. The pharynx epithelium cells are round, with large, round, deeply-staining nuclei. The cells of this regenerating epithelium can be traced to continuity with the actively regenerating brain and the cells of the former are indistinguishable cytologically from the cells at or near the periphery of the regenerating cerebral ganglia. Farther within the brain these cells become progressively transformed into the large, characteristic nerve cells of this organ. The external epithelium of the body wall is newly regenerated, but it shows no activity anywhere involving the movement of cells towards the regenerating brain. At this stage the cerebral ganglia are of very nearly normal size.

In the specimen illustrated the regenerating dorsal pharynx wall is itself of ectodermal derivation and has not yet taken on its ultimate morphological and functional specialization. However, it is possible for the adult fully differentiated pharynx to form a new brain as was shown by Nuzum and Rand. In the present work the dorsal pharynx wall was injured but the last cited work demonstrated that such injury is not essential to cerebral ganglia formation.

6948

Relationship of Bacteriophage to Toxin and Antitoxin.

N. W. LARKUM.

From the Bureau of Laboratories, Michigan Department of Health.

Some investigators have implied that bacteriophage action was identical to the action of toxin and antitoxin. d'Herelle suggested that bacteriophage was in fact an antitoxin. The observations on the clinical application of bacteriophage have awaited an adequate theoretical basis before they could be interpreted and a foundation laid for intelligent formulation of a rationale of treatment and prophylaxis. Staphylococcus toxin has only recently been developed to a point where it may be safely assumed that such a toxin exists, and where means of measuring its activity and potency have been sufficiently standardized to permit of fruitful study. Having then a satisfactory staphylococcus toxin and a bacteriophage highly active against staphylococci, the necessary elements for a study of the relationship between toxin and bacteriophage have become available. The following report is a preliminary study of this relationship from which it is believed definite conclusions can be drawn.

The production of staphylococcus toxin followed the method given by Dollman[1] and based upon the earlier work of Burnet[2, 3] and his coworkers. Plates of beef extract broth to which 0.5% agar had been added were inoculated with staphylococcus and incubated for 48 hours in an atmosphere of 15% CO_2. The semi-fluid material after incubation was passed through filter paper, the reaction adjusted to pH 6.6 and 1:8000 merthiolate was added. Six toxins were prepared in this manner and their potency tested first by hemolysis of rabbit red blood cells. The results are shown in Table I.

Bacteriophage itself incubated under similar conditions with rabbit red blood cells produced no hemolysis and hence has none of the hemolytic properties of staphylococcus toxin.

Neutralization of staphylococcus toxin with bacteriophage was attempted by incubating 0.1 cc. of toxin with bacteriophage undiluted in the following amounts, 0.1 cc., 0.2 cc., and 0.5 cc. of phage. Toxin in dilutions of 1:50 and 1:100 was also incubated with similar amounts of bacteriophage. In no case was there any inhibition of hemolysis.

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[1] Dollman, E. C., *Canadian Public Health J.*, 1932, **23**, 125.

[2] Burnet, F. M., *J. Path. and Bact.*, 1929, **32**, 717.

[3] Burnet, F. M., *J. Path. and Bact.*, 1930, **33**, 1.

TABLE I.

Toxin dilution 1:50.
Rabbit red blood cells, 1% suspension. 0.2 cc. in each tube.
Total volume .4 cc., tubes 85 x 9 mm.

Toxin	.2 cc.	.1 cc.	.06 cc.	.04 cc.	.02 cc.	.01 cc.
1909†	++++	++++	++	+	—	—
1968	++++	++++	+	—	—	—
1908	++	—	—	—	—	—
1910	++++	—	—	—	—	—
1904	++++	++++	++	+	+	—
1912	++++	—	—	—	—	—
	1:100	1:200	1:333	1:500	1:1000	1:2000

++++ Complete hemolysis.
+ Supernatant fluid colored.
— Colorless supernatant.
Incubation, 1 hour 37° in water bath.
†These cultures were all susceptible to bacteriophagy and have been used in preparation of bacteriophage for therapeutic use.

Two rabbits were bled and the serums tested for antitoxic properties. Neither serum inhibited the hemolytic power of the toxin used (1904). One rabbit was then given 2 intravenous injections of toxin 1:50 dilution, 0.2 cc. followed by 0.5 cc. 24 hours later. The other rabbit was given 2 injections of bacteriophage, 2.0 cc. followed by 2.0 cc. after 24 hours. Both animals were bled after 5 days and the serum tested for antitoxin. The results are shown in Table II.

TABLE II.

Toxin dilutions 1:10, 1:25, 1:50, 1:100, 1:200, 1:400.
0.1 cc. of toxin dilution + 0.1 cc. of serum incubated 1 hour at room temperature. 0.2 cc. 1% rabbit cells added, incubation 1 hour at 37° in water bath.

Final toxin dilution	1:40	1:100	1:200	1:400	1:800	1:1600
Serum 733*	+++	++	+	—	—	—
" 737*	++	—	—	—	—	—
No serum	++++	++++	++++	+	—	—

*Serum 733 Produced by injections of toxin
" 737 " " " " bacteriophage
Toxin 1904

This test was repeated with various dilutions of serum and toxin but the results presented here are typical.

Staphylococcus toxin is not only hemolytic; it has strong dermonecrotic properties, and in order to check the above results, a series of skin tests were performed on rabbits. Staphylococcus toxin when injected intradermally into rabbits gives a skin reaction that consists at first of an area of erythema later deepening and having a necrotic center. The readings in this case were made after 70 hours and the size of the reaction zone was measured. The dermonecrotic titre is usually less than the hemolytic titre with a given serum. Toxin 1904 gave a measurable area in 1:20 dilution and visible reactions up to 1:80. Undiluted antitoxic serum from the rabbit immunized with bacteriophage neutralized the toxin in 1:10 dilution. When the antitoxic serum from the same rabbit was diluted 1:2 it still neutralized the toxin in 1:10 dilution. In 1:40 dilution this serum failed to neutralize. The serum itself gave a very faint skin reaction. The antitoxin produced by inoculating a rabbit with the staphylococcus toxin failed to neutralize the dermonecrotic toxin even when the serum was undiluted.

As bacteriophage antiserum was definitely antitoxic for staphylococcus toxin, the question arose as to whether the bacteriophage itself was responsible for the production of the antitoxin or whether there were in the bacteriophage filtrate other substances capable of producing the antitoxin. Bacteriophage was purified according to the method described by Krueger,[4] *i. e.*, electrophoresis. A purified phage was produced containing less than 0.1 mg. of nitrogen per cc. and this product injected into a rabbit, two 5 cc. intravenous injections being given, the second 48 hours after the first. The rabbit was bled after 10 days and the serum tested for antitoxin. The results are shown in Table III.

TABLE III.

Final toxin dilution	1:20	1:40	1:80	1:160	1:320
Toxin	++++	++++	+++	+	—
Toxin and serum 733	++++	++	+	—	—
Toxin and serum 736	++++	+	—	—	—

Serum 733 Produced by injection of toxin
" 736 " " " " purified bacteriophage

It appears from this experiment that the purified bacteriophage produced an antitoxin comparable to that produced by injection of unpurified phage and hence that the bacteriophage itself is the antigen concerned with the production of staphylococcus antitoxin.

It appears reasonably certain that bacteriophage when used as an antigen produces an antiserum capable of neutralizing staphylococcus toxin and that it is the bacteriophage itself which has this property. Bacteriophage is not a toxin or antitoxin, and is apparently in the nature of a toxoid.

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[4] Krueger, A. P., and Tamada, H. T., *J. Gen. Physiol.*, 1929, **13**, 145.

6949

Comparative Actions of Dextro- and Levo-Epinephrine on Human Heart.

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Shortly after the synthesis of epinephrine, studies were carried out on the comparative actions of the synthetic and the natural substances. Chemically the artificial base was identical with the natural substance in all respects except that it was optically inactive while natural epinephrine is levorotatory. Beberfeld[1] studied the comparative actions of racemic synthetic epinephrine and the levorotatory natural base in rabbits and concluded that they were exactly equivalent. Cushny[2] found in dogs that the natural epinephrine was twice as active on the blood pressure as the synthetic isomer and concluded that the dextrorotatory substance was devoid of action on the blood vessels. Since 1908 when the dextro-component became available, several observers have reported on the comparative activities of the isomers. Practically all agree that the d-isomer has a relatively weak action. The following ratios of activity have been reported in dogs: 1:12 to 1:15, by Cushny[3]; 1:15 by Abderhalden and Müller[4]; 1:15 to 1:20 by Tiffeneau[5]; and 1:30 to 1:40 by Fromherz.[6] Richaud,[7] however, concluded that levo-epinephrine was only slightly more active than the racemic form so that in therapy the synthetic substance should give approximately the same effect. His method, however, for estimating quantitative reactions is not entirely acceptable. In rabbits Launoy and Menguy[8] found a ratio of activity of 1:30, while in cats Tainter[9] reported a ratio of 1:20.

The method which has been used almost exclusively for the study of comparative actions of these isomers has been the pressor response in experimental animals. Cushny[3] has pointed out that the comparative activities of optical isomers may not be the same on different organs. For example, he observed that in its peripheral action levo-hyoscyamin was 12 times as active as the dextro-isomer. He noted, however, that certain tissues prove exceptions to the general rule, as on the central nervous system d-hyoscyamin acted quite as strongly as the levorotatory substance.

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[1] Beberfeld, J., *Med. Klin.*, 1906, **2**, 1177.

[2] Cushny, A. R., *J. Physiol.*, 1908, **37**, 130.

[3] Cushny, A. R., *J. Physiol.*, 1909, **38**, 259.

[4] Abderhalden, E., and Müller, F., *Z. f. Physiol. Chem.*, 1908, **58**, 185.

[5] Tiffeneau, M., *Compt. Rend. de Acad. des Sciences*, 1915, **161**, 36.

[6] Fromherz, K., *Deutsch. med. Woch.*, 1923, **49**, 814.

[7] Richaud, A., *J. de Pharm. et Chem.*, 1922, **25**, 369.

[8] Launoy, L., and Menguy, B., *Compt. Rend. Soc. de Biol.*, 1922, **87**, 1066.

[9] Tainter, M. L., *J. Pharmacol. and Exp. Therap.*, 1930, **40**, 43.

The purpose of the present study was to determine whether the quantitative differences exhibited by the epinephrine isomers on the blood vessels of experimental animals apply to the human heart. It was previously demonstrated that natural epinephrine consistently abolished the cardiac standstill which may be induced in many human subjects by pressure on the right carotid sinus.[10] The standstill is abolished by the formation of secondary centers of impulse initiation in the ventricles. By applying varying doses of epinephrine intravenously it was observed that the rate of the ectopic pacemaker and the duration of the effect were proportional to the

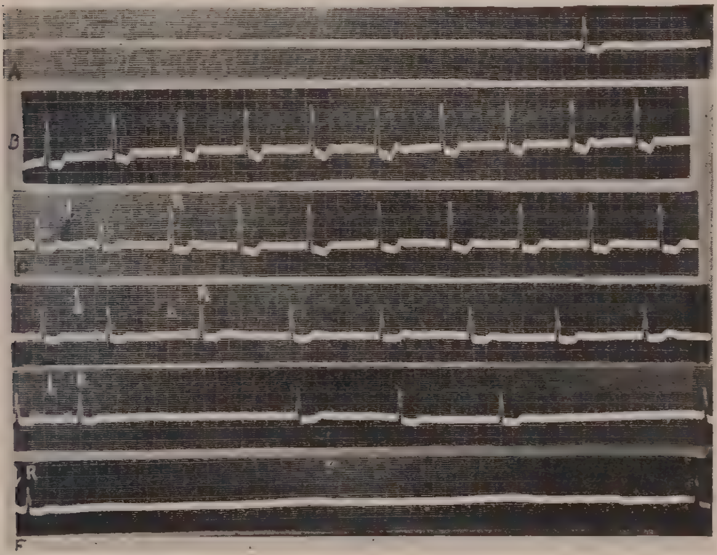

FIG. 1.

Strip A shows response to pressure on the right carotid sinus (arrow), cardiac standstill of 7.6 seconds. Lower strips show reaction to right carotid sinus pressure 2 minutes, 5 minutes, 10 minutes, 12 minutes and 15 minutes after the intravenous injection of 1/20 mg. of l-epinephrine.

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[10] Nathanson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 967.

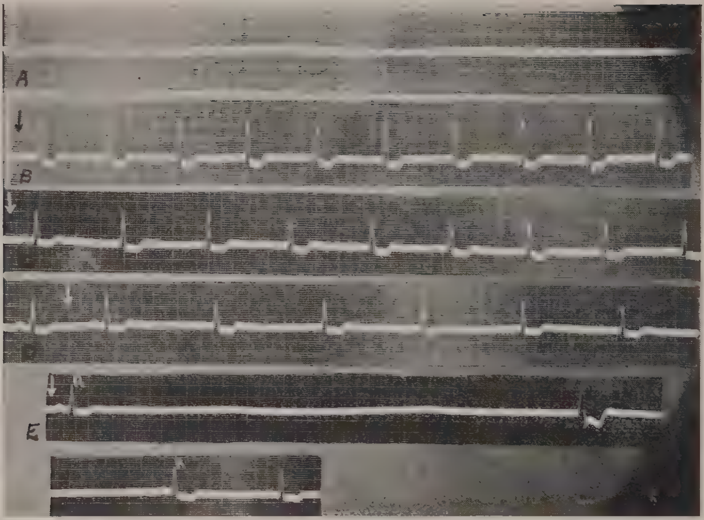

FIG. 2.

Strip A shows standstill of 11.8 seconds on right carotid sinus pressure. Lower strips show reaction to right carotid sinus pressure 2 minutes, 5 minutes, 10 minutes and 15 minutes after the intravenous injection of 1 mg. of d-epinephrine.

administered dose. Fig. 1 illustrates the response in one subject to 1/20 mg. of l-epinephrine intravenously showing a maximum rate of the ventricular pacemaker of 60, and the duration of effect of 15 minutes. A dose of 1/5 mg. produces a more rapid ventricular rate of longer duration while the response to 1/50 mg. is a slow ventricular rhythm of a shorter duration, 8 to 10 minutes. Repeated administration of these doses gave consistent responses. The reaction to d-epinephrine was studied on 3 occasions.* In each experiment 1 mg. of d-epinephrine produced a reaction practically identical in intensity and duration with that obtained by 1/20 mg. of l-epinephrine, thus giving a ratio of activity of 1:20. These experiments indicate that the human ventricle discriminates between the epinephrine isomers in the same manner as has been previously demonstrated for the blood vessels of experimental animals.

The pressor reactions to both isomers showed the same quantitative differences. In each case the blood pressures were between 140 and 150 systolic and 80 to 90 diastolic before the injection. After the injection of 1/20 mg. of l-epinephrine the pressure rose to 198 systolic and 110 diastolic. The administration of 1 mg. of d-epinephrine produced a rise in systolic pressure to 190 and in the diastolic to 110. The blood pressures returned to the original level within 3 minutes with both substances. With both isomers the duration of the cardiac effect was 5 times that of the pressor reaction.

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* The d-epinephrine was supplied by the H. A. Metz Laboratories as the bitartrate. For purposes of comparison with the l-epinephrine, which was in the form of hydrochloride, the doses are expressed in terms of epinephrine base.

6950

Iodine Content of Human Skim Milk from Goitrous and Non-goitrous Regions.

R. G. TURNER. (Assisted by M. Z. Weeks.)

From the Department of Medical Research, Detroit College of Medicine and Surgery.

Published figures for the iodine content of cow's and women's milk are not consistent. The foreign investigators[1] report for cow's milk, 1γ-3γ% and for human milk after the fifth lactation day 2γ-4γ%. American investigators[2] find that the percentage in cow's skim milk, calculated on the dry basis, may vary between 14.0γ and 130.0γ%.

This investigation includes a study of the iodine content of the milk from mothers in Detroit, a goitrous region, and from mothers in Boston, a non-goitrous region. The mothers were normal and showed no signs of thyroid hyperplasia. / The value of iodine was determined in the milk of mothers at varying intervals during the mature lactation period. The calcium and inorganic soluble phosphates were also determined and compared with the fluctuations found for iodine.

The milk was obtained through the courtesy of the Detroit Mother's Milk Bureau and The Directory for Mother's Milk, Boston. Nine cases were studied from the former and 10 cases from the latter.

Micro methods were used for the determination of calcium, inorganic soluble phosphates, and iodine. Samples of one ounce were received and a 10 cc. portion of this was needed for the micro-iodine estimation, as described by the author.[3, 4] The calcium and inorganic soluble phosphates were determined on the trichloracetic acid filtrates from 2 cc. of skimmed milk. Calcium was estimated by the method of Clark,[5] the inorganic soluble phosphates by the method of Benedict and Theis.[6] The results represent analyses of 295 samples of milk.

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[1] Maurer, E., and Diez, S., *Munch. Med. Woch.*, 1926, **73**, 17; von Fellenberg, T., *Ergebn. Physiol.*, 1926, **25**, 176; Maurer, E., and Ducrue, H., *Munch. Med. Woch.*, 1928, **75**, 249.

[2] McClendon, F., Remington, R. C., and von Kolnitz, H., *J. Am. Chem. Soc.*, 1930, **52**, 541; 1931, **53**, 1245.

The average iodine content of human skim milk may vary among women and at different times during the lactation period from 6.0γ to $23.0\gamma\%$. The average is 12.4γ. There is a marked variation in individuals. The average iodine value is higher throughout the lactation period in a goitrous region than in the non-goitrous region, averaging 14.5γ and $12.4\gamma\%$, respectively. The percentage difference is greatest in the early months of lactation approaching the same level after the eighth month with a percentage difference of only 2.9 in the tenth lactation month. The monthly averages of all cases are plotted in Chart 1. Omitting case 5, Detroit, (used iodized salt daily) the iodine curve more closely resembles that of the Boston group, but the average value continually remains higher.

The calcium and inorganic soluble phosphate curves run parallel for each group investigated. The milk from the Detroit mothers is more concentrated in both these constituents during the early months of lactation. The values for both drop continually to the fourth month where they reach approximately the same level as the Boston group, then gradually fall below this level. In general the results show that the iodine content of human milk follows the trend found for calcium and phosphorus, decreasing in concentration after the third or fourth lactation month to the end of the mature lactation period.

These findings represent a compensatory action on the part of the physiological mechanism of the mother from the goitrous region to supply a greater amount of iodine to the child than is naturally supplied in the milk of mothers from a non-goitrous region. Else[7] states that if the thyroid gland of a mother is not secreting enough thyroxin during pregnancy a demand is thrown upon the child's gland to function. This causes a secretion of colloid which results

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[3] Turner, R. G., *J. Biol. Chem.*, 1931, **88**, 497.

[4] Turner, R. G., and Weeks, M. Z., *J. Am. Chem. Soc.*, 1933, **55**, 254.

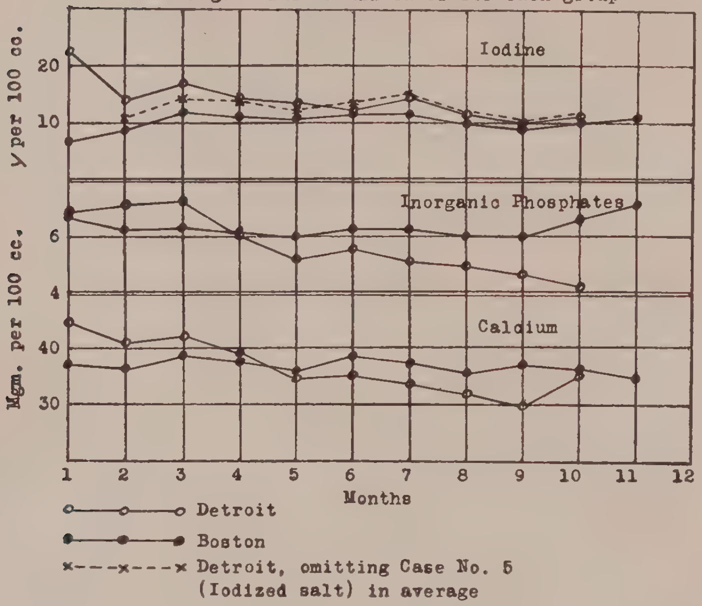
[5] Clark, G. W., *J. Biol. Chem.*, 1921, **44**, 487.

[6] Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, **61**, 63.

[7] Else, J. E., *Bull. Assn. Study Intern. Secret.*, 1929, **13**, 40.

Chart 1. Iodine, Inorganic Phosphates, and Calcium

Average value of all cases for each group



in a colloid goiter. If enough iodine is not supplied in the mother's milk, this same demand may be made upon the lactating infant's gland./ The perfectly normal thyroid gland receiving sufficient amount of iodine will not develop goiter. Thus the theory of the compensating act of the mother to provide more iodine for an infant born in a non-goitrous region is reasonable as well as justified by the actual findings in this research. This further shows that the natural prevention of goiter in an individual born with a normal thyroid is largely that of supplying a greater amount of iodine in its diet.

6951

Blood Regeneration in Anemic Rats on a Vitamin G-Deficient Ration.*

CHARLES J. STUCKY AND ERWIN BRAND.

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Pellagra is frequently complicated by anemia during the advanced stages.[1] One investigator[2] suggested that the disease may be due to an iron-deficiency. However, Halliday failed to cure vitamin G deficiency in her rats by means of iron therapy.[3] Recently it was reported that an anemia could be produced in rats in which vitamin G deficiency was accompanied by skin lesions.[4] Accordingly, experiments were undertaken to determine the effect of a vitamin G deficient ration on hemoglobin regeneration in anemic animals.

A group of rats of the Wistar strain, reared as previously described,[5] was employed. When weaned (21 days) such animals have a lower Hb level (6.0 gm.) than our normally bred rats[6] (av. Hb = 8.0 gm.). The animals were housed individually in wire cages with false bottoms and fed the following vitamin G deficient, iron-containing ration: corn starch 61, casein (vitamin-free) 20, lard 10, cod liver oil (Squibb) 5 and salt mixture (Osborne and Mendel) 4%. Each animal received daily a supplement of 0.2 pigeon unit[7] of a vitamin B_1 (anti-neuritic) concentrate.† Hemoglobin was estimated on tail blood at weekly intervals.[6]

Results. None of the rats (8 animals from 2 litters) made significant gains in body weight and all declined before the end of the experimental period (approx. 1 month). No skin lesions were observed in these rats. In contrast to the failure in growth, there was a rapid increase in the Hb in all cases until normal levels (av.

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* A preliminary report of this paper was presented before the Division of Biological Chemistry of the American Chemical Society, Washington, D. C., March 29, 1933.

[1] Turner, R. H., *Am. J. Med. Sci.*, 1933, **185**, 381.

[2] Bliss, S., *Science*, 1930, **72**, 577.

[3] Halliday, N., *Science*, 1931, **74**, 312.

[4] Sure, B., Kik, M. C., and Smith, M. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 498.

[5] Stucky, C. J., *J. Biol. Chem.*, 1932, **97**, xiii.

[6] Stucky, C. J., and Brand, E., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 932.

† Kindly furnished by Dr. Richard J. Block.

[7] Block, R. J., Cowgill, G. R., and Klotz, H. B., *J. Biol. Chem.*, 1932, **94**, 765.

Hb = 15.5 gm.) were reached. Our stock animals of the same age show similar Hb values[6] (av. = 15.0 gm.).

From these data it would appear that vitamin G deficiency is not due to an iron-deficiency. The results confirm the reports of other investigators.[3, 4]

Summary. Young anemic rats were fed a vitamin G deficient (iron-containing) ration for approximately one month. The animals failed to grow but recovered from their anemia in 3 to 4 weeks.

6952

Methods and Results of Barbital Research.

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From the Department of Pharmacology and Materia Medica, Georgetown University School of Medicine.

We described[1] a colorimetric test for barbiturates and the rate of excretion of barbital determined by this test. We here report on (a) urinary elimination, (b) fate in the blood, (c) presence in organs of barbiturates, and (d) on the clinical application of this test.

The progress in these studies was made possible by the colorimetric test and by improved methods of extraction of barbiturates, consisting of shaking urine (alkalinized), oxalated whole blood, plasma or spinal fluid with equal volumes of 10% copper sulphate solution, filtering, acidulating the filtrate with dilute sulphuric acid and shaking the filtrate with 10 volumes of chloroform. The chloroform extract may be concentrated on water bath. Ground organs, before they are shaken with copper sulphate must be liquefied, either by 3% HCl and pepsin, or by mixing thoroughly with 5% KOH. Heating must be excluded in these procedures. The acid-pepsin treated organs must be alkalinized before shaking with copper sulphate.

Results. (a) *Urinary Excretion.* The excretion of barbital was studied in normal dogs (70-300 mg. per kg., intravenously), in the cat (250 mg. per kg., intravenously), humans (30 grains total, by mouth) and in the fowl (225 mg. per kg., intravenously). Normal dogs (8), cat (1), and humans (2) excreted from 42% to 89% of barbital during 7 days (more than ½ of the dose usually in the first 48 hr.), from 13% to 16% of phenobarbital (2 dogs, 100 mg. per kg., intravenously) and about 40% each of neonal (1 dog, 60 mg. per kg., intravenously) and dial (1 dog, 80 mg. per kg., intravenously) and only small amounts of pernoston and amytal (3 dogs). Diuretic treatment (intravenous injections of 50 cc. of 10% glucose solution per kg. of body weight) did not increase the percentage excretion of barbiturates (from 50% to 92% for barbital (5 dogs), 16% for phenobarbital (1 dog), and 31% dial (1 dog, 100 mg. per kg., intravenously). One chicken behaved atypically, it excreted only 28.4% of barbital and never recovered completely.

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[1] Koppanyi, Murphy, and Krop, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 542.

One dog with the left kidney removed, excreted 69% of barbital (225 mg. per kg., by vein) during a period of one week.

(b) *Fate in Blood.* All doses of barbital in the animal experiments were administered intravenously in the form of the soluble sodium salt, which changes to diethyl-barbituric acid in the blood, even if Na Barbital is added to drawn blood in the test tube. This fact was demonstrated by recovering all of the hypnotic from the blood without acidulation. The ratio of the amount of barbital taken up by the plasma to that taken up by the corpuscles is approximately 3 to 1.

The concentration of barbital in the blood was studied in normal and bilaterally nephrectomized dogs. Following the intravenous injection of the minimum anesthetic dose (225 mg. per kg.) in normal dogs, the following determinations were made (typical experiment): 1 minute, 0.4 mg. per cc. blood; 3 minutes, 0.375 mg.; 5 minutes, 0.3 mg.; 10 minutes, 0.25 mg.; 30 minutes, 0.225 mg.; 1 hr., 0.15 mg.; 4 hr., 0.12 mg.; 6 hr., 0.075 mg.; 24 hr., 0.018 mg. Following the intravenous administration of the same dose in bilaterally nephrectomized dogs, the following figures were obtained: 2 hr., 0.15 mg. per cc. of blood; 3 hr., 0.15 mg.; 4 hr., 0.15 mg.; 20 hr., 0.15 mg.; 40 hr., 0.15 mg. Other barbiturates were detected in the blood in far smaller amounts. The bilaterally nephrectomized dogs maintaining a constant barbital level in the blood never recovered from the anesthesia and eventually died. However, when bilaterally nephrectomized dogs were anesthetized with neonal, nembutal, or pernoson, they recovered from the anesthesia.

(c) *Presence in Organs.* The presence and concentration of barbital in organs was studied in dogs and cats. We submit 2 typical cases:

Dog, 8.23 kg., 225 mg. sodium barbital per kg. intravenously. Twenty-four hours after injection.

Liver (145.0 gm.)—75.0 mg. total
Muscle (138.0 gm.)—22.5 mg. total
Brain (71.0 gm.)—24.88 mg. total
Heart (65.2 gm.)—27.0 mg. total
Saliva (45.0 cc.)—20.0 mg. total

Kitten, 0.670 kg. 400 mg. sodium barbital per kg. intravenously. One hour after injection:

Heart (2.13 gm.)—1.0 mg. total
Kidneys (2.8 gm.)—3.37 mg. total
Liver (24.75 gm.)—14.4 mg. total
Brain (19.13 gm.)—3.75 mg. total
Spleen and pancreas (8.3 gm.)—2.25 mg. total
Muscle (33.2 gm.)—21.41 mg. total

Barbital was also recovered in the cerebro-spinal fluid and in the amniotic fluid and embryos of pregnant dogs.

In conclusion we might say that barbital is almost evenly distributed in the different organs without concentrating in any particular tissue.

(d) *Clinical Applications.* Poisoning with barbital is frequently encountered clinically, and during the brief span of 2 months, 5 cases were referred to us. In each case we were able to diagnose the poisoning by urine analysis. For example, 95 cc. of urine of Mr. H. G. (Gallinger Municipal Hospital) contained 383.0 mg. barbiturate. Subsequent investigations revealed that the patient had taken 6 tubes(?) of veronal tablets. Mr. P. (Gallinger Municipal Hospital) was transferred to the hospital in comatose condition. Two catheterized specimens of urine contained a total of 298.0 mg. barbiturate. At the same time, 0.15 mg. of barbiturate was found per cc. of blood.

6953

Inhibition of Diuresis by Hypnotics.

ROBERT P. WALTON. (Introduced by J. T. Halsey.)

From the Department of Pharmacology, Tulane University, School of Medicine.

The depression of diuretic function by anesthetics or hypnotics has often been attributed to certain effects peculiar to the type of anesthetic under consideration. Dooley and Wells[1] have suggested that ether anuria may be explained as a reflex arising from pulmonary irritation. Pick and collaborators[2, 3, 4, 5] have reported widely varying results with different hypnotics, some increasing diuresis in contrast to the more usual depression of function. Experiments in this laboratory, on the other hand, have indicated a more uniform depression of diuresis and have suggested that the effect is also associated with the condition of anesthesia in general. The chief exception to such a view is the reported diuretic effect of paraldehyde. The most prominent of these reports dealing with the effect on dogs is that by Bonsmann,[5] who used 9 different hypnotics and obtained results varying widely between the 2 extremes represented by paraldehyde and sodium luminal. The effect of these 2 drugs on dogs has been reinvestigated in this study, approximately according to a procedure previously described by the writer.[6] This requires a regularity in diet and a normal diuretic capacity for 2 or more days immediately preceding the day of experiment.

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[1] Dooley and Wells, *Am. J. Physiol.*, 1929, **90**, 330.

Orally administered doses of paraldehyde ranging from 0.75 cc. to 2.0 cc. kg. depressed the diuretic function to 24% of the normal for the 2-hour period following oral administration of water, 20 cc. kg. (av. of 15 exp.; lowest, 11%; highest, 45%). Diuresis with doses of 2.0 cc. kg. corresponded to 19% of the normal (av. of 6 exp.; lowest, 11%; highest, 29%). At this dose a dog is not ordinarily able to lift its head in 10 min. and does not recover this ability for 8½ hours. Surgical anesthesia is frequently produced for about 1 or 2 hours but is more often complicated by motor excitation.

With sodium luminal, orally administered in doses ranging from 50 to 150 mg. kg., diuresis for the same period was depressed to 11% of the normal (av. of 9 exp.; lowest, 2%; highest, 20%). Dosages above 100 mg. kg. were fatal. True surgical anesthesia was not obtained in the first few hours of any of these experiments.

For a corresponding 6-hour period, diuresis averaged about 62% of the normal with paraldehyde and 30% with sodium luminal. These differences are by no means as great as those reported by the Vienna school of pharmacologists but are nevertheless distinct differences.

In an effort to separate drug effects from anesthetic effects, these depressant drugs were antagonized with the stimulant drugs, Metra-

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[2] Molitor, H., and Pick, E., *Arch. Exp. Path. Pharm.*, 1925, **107**, 180, 185; 1926, **112**, 114; *Biochem. Z.*, 1927, **186**, 130.

[3] Kugel, M. A., *Arch. Exp. Path. Pharm.*, 1929, **142**, 166.

[4] Buschke, F., *Arch. Exp. Path. Pharm.*, 1928, **136**, 43.

[5] Bonsmann, H. R., *Arch. Exp. Path. Pharm.*, 1930, **156**, 160.

[6] Walton, R. P., *J. Pharm. Exp. Ther.*, 1933, **47**, 141.

zol and picrotoxin. Overdosage of the stimulants either had no effect or caused greater depression of diuresis. Properly balanced dosages of the antagonists, however, frequently increased diuresis to a marked degree over that observed with the depressant alone and most frequently in those cases where the degree of depression was lessened.

Paraldehyde was used throughout at dosages of 2.0 cc./kg. Eight experiments with Metrazol indicated that 35 mg./kg. intraperitoneally was the most suitable dosage for this antagonist. By injection a few minutes before the paraldehyde, the period of depression was delayed 30 to 40 minutes but the time of recovery was not particularly affected. By injection a few minutes after the paraldehyde, there was no particular effect on the time of appearance of the depression but the period was considerably shortened. Two experiments with picrotoxin (2 to 3 mg./kg.) increased diuresis over that with paraldehyde alone but was not a suitable antagonist at this dosage because of the convulsions produced.

Three experiments were made with sodium luminal at dosages 110 to 150 mg./kg. Diuresis was increased during the period of stimulation but the effect on recovery was unfavorable, apparently because of the unusually prolonged action of this drug.

6954

Attachment of Marine Bacteria to Submerged Slides.

C. E. ZOBELL and ESTHER C. ALLEN. (Introduced by M. L. Tainter.)

From the Scripps Institution of Oceanography, University of California, La Jolla.

Winogradsky,[1] Cholodny,[2] and Conn[3] have shown that a different bacteriological picture is presented when the microorganisms which attach themselves to glass slides incubated in the soil are observed directly, than when the same soil is studied microbiologically by the conventional cultural methods. Henrici[4] studied freshwater bacteria by such a direct microscopic method. We used a somewhat similar procedure to study marine bacteria on the coast of southern California.

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[1] Winogradsky, S., *Soil Sci.*, 1928, **25**, 37.

[2] Cholodny, N., *Arch. f. Mikrobiol.*, 1930, **1**, 620.

[3] Conn, H. J., *Z. f. Bakt.*, 1932, **87**, 233.

[4] Henrici, A. T., *J. Bact.*, 1933, **25**, 277.

Our experiments were designed to supplement the work of Coe and Allen (Coe[5]) at the Scripps Institution of Oceanography on "fouling organisms" so destructive to navigation equipment and other marine structures (Visscher[6]).

Standard slides, bacteriologically clean, were submerged off the end of Institution pier, and after one to 7 days' submergence the slides were taken with aseptic precautions to the laboratory for examination. Results indicate that attachment of bacteria and, to a lesser extent, diatoms and actinomycetes, usually precedes the attachment of barnacles, hydroids, bryozoa, and other "fouling organisms" by several hours or even days.

Many forms of bacteria which have not been recovered by concurrently plating samples of sea water on culture media have been observed directly on these slides. However, not all marine bacteria attach themselves to submerged glass slides.

Seventy-three pure cultures isolated from the sea and differing morphologically or physiologically were tested under controlled conditions for their attachment propensities. The bacteria were inoculated into wide-mouth bottles containing nutrient sea water. Sterile glass slides were inserted vertically. After 2 days' incubation at 25° C. the slides were examined. With no fixation the slide was stained and dried. Only 24 of the 73 cultures attached themselves to the slides, although those which did not attach had multiplied until the medium had become turbid. The bacteria which had firmly attached to slides will be termed "attachment bacteria". Some of them appeared to be definitely thigmotactic because they grew only in adherent films on surfaces. Among the 24 attachment bacteria 3 such thigmotactic cultures formed macroscopically visible films on bottles and slides before the broth itself became perceptibly turbid. On the other hand, in some pure cultures enough cells attached themselves to the slides to warrant being classified as "attachment bacteria" although the sea water broth was very cloudy with free-floating cells.

Only some marine bacteria attach themselves to submerged slides. The direct microscopic technic is not applicable to a quantitative study of marine bacteria because the submerged slides will detect only the bacteria which attach themselves to surfaces, and these seem to be in the minority. Incidentally, if the same relationship exists among soil or fresh-water bacteria, this would have to be considered in the interpretation of results obtained by the Cholodny-Conn technic.

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[5] Coe, W. R., *Bull. Scripps Instit. Oceanog., tech. ser.*, 1932, **3**, 37.

[6] Visscher, J. P., *Bull. Bur. Fish.*, 1927, **43**, 193.

It usually requires 2 to 4 hours for appreciable numbers to fix themselves to slides firmly enough so that they will not be washed off by running water and the staining processes. Thus, the submergence of a slide in broth already cloudy with bacteria followed by its immediate removal does not result in the attachment of bacteria. Under these conditions a few cells of both the attachment, as well as the non-attachment types, may accidentally adhere to the slide, but the number is small and the arrangement irregular. Attachment occurs more readily during the logarithmic growth phase than during the phase of negative acceleration or later. The best preparations of attachment bacteria are obtained by inserting the slides at the same time the media are inoculated, or shortly thereafter.

All stages of bacterial development from individual cells, dividing cells, chains, and micro-colonies have been observed. It is quite evident that many of them are multiplying on the surface of the slide. Hence, such adherent films are invaluable for observing the stages in the processes of cell division or multiplication, because the intricate details are not disrupted as is frequently the case in the preparation of smears in the usual way. Thus, 2 forms have been noted which, though otherwise indistinguishable from the bacilli, were found to multiply by fragmentation actinomyces-like.

Most, if not all, of the attachment bacteria are capsulated. Some of them appear to develop a definite holdfast, although it has been difficult to find a way to ascertain the nature of the attaching mechanism. Certain other forms produce a film of faintly staining material extending beyond the cells 2 or 4 times their diameter, and on this the bacteria are grouped making them appear to be arranged on islands of this material. The attachment tendency has been found to be affected by the composition of the media, glucose favoring it. As stated above, water does not destroy the holdfast. Neither is it destroyed by alcohol, but xylol removes many of the attached cells.

6955

Effect of Sodium Cyanide on Complement Hemolysis.

W. H. RANDALL. (Introduced by M. S. Fleisher.)

From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

The addition of various amounts of neutralized NaCN to the complex, red cells, specific hemolysin, and complement partially or completely inhibits hemolysis. (0.4 cc. or 0.5 cc. of 1% solution of NaCN in a total volume of 3 cc.) It makes no difference whether the cyanide is incubated first with the amboceptor or with the complement, for on the addition of the other reagents of the test the same degree of inhibition is noted.

The inhibitory action is not due to the HCN gas liberated by neutralization of the alkaline NaCN. The NaCN solution was adjusted to pH 7 by addition of HCl. This was tested by saturating all of the constituents of the test with HCN gas and comparing the time necessary for complete hemolysis, by reagents saturated with HCN gas with the time required by untreated reagents. The time was the same in both cases showing that the HCN gas was not inhibitory.

The cyanide does not effect the union of immune hemolysin with the red cells as washing the red blood cells after contact with hemolysin in the presence of cyanide in order to remove the cyanide, permits hemolysis to take place when complement is added.

These results are contradictory to those of L. Jarno and L. Suranyi,[1] who suggest that the action of NaCN is due to the inactivation of a cholic acid-like complex which they believe is associated with the properties of immune hemolysin.

Inasmuch as these workers fail to state whether they neutralized the cyanide their results might be due to the alkalinity of the cyanide solution. Experiments were carried out to show that the alkalinity of NaCN is sufficient to inactivate complement when used in amounts identical with those reported by the above workers. When unneutralized NaCN is used as above, the pH of the mixture was 11, but the union of amboceptor with red cells is not affected, as after washing red cells which have been treated with NaCN treated amboceptor, the addition of fresh complement results in hemolysis. On the other hand the presence of the unneutralized NaCN inhibits the action of the complement in mixtures of red cells, hemolysin and complement.

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[1] *Z. für Immunitats.*, 1930, **69**, 298.

Apparently the effect of the cyanide in inhibiting hemolysis is upon the complement and not upon the antigen-amboceptor complex: and this effect is not dependent upon the pH of the cyanide.

6956

Observations on Serological Reactions with Albumose Preparations. II.

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In a former communication[1] reactions with immune sera produced by injections of heteroalbumose preparations were briefly described. In order to give some additional information the following experiments, which were carried out with 3 albumose fractions obtained from the products of peptic digestion of coagulated sheep serum, may be cited.

Heteroalbumose was prepared in the manner previously described.[2] The preparation was precipitable from 1% saline solution by about 33% alcohol or by one-half volume of saturated ammonium sulfate solution.

After removal of heteroalbumose, the albumoses which were precipitated between ½ and ⅔ saturation with ammonium sulfate were further fractionated at icebox temperature by precipitation with 65% alcohol at pH 5.1, fraction 1 being insoluble, fraction 3 remaining in solution. Fraction D 1 was precipitable by alcohol in a concentration above 50%, or by addition of 1 to 1.25 volumes of saturated ammonium sulfate solution. Fraction D 3 was precipitable by alcohol in a concentration above 80%, or by addition of 1.25 to 1.5 volumes of saturated ammonium sulfate solution.

Precipitin tests with a particularly strong antiserum obtained by injections of crude heteroalbumose mixed with charcoal[3] are given in Table I.

As already stated, by means of inhibition tests reactions could be

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[1] Landsteiner, K., and van der Scheer, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 983.

[2] Landsteiner, K., and van der Scheer, J., *Z. Hyg. u. Infekt.*, 1931, **113**, 2.

[3] Landsteiner, K., and Jacobs, J., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 570.

TABLE I.

Precipitin Tests.

Two drops of immune serum were added to 0.2 cc. of the albumose solutions. Dilutions are given in terms of 5% stock solutions. The intensity of the reactions is indicated as follows: 0, fftr. (very faint trace), ftr. (faint trace), tr. (trace), ±, +̲, +, + ±, etc.

		Dilution 1:									
	Reading after	100	250	500	1000	2500	5000	10000	20000	50000	100000
Heteroalbumose	½ hr.	±	+	+ ±	+ ±	+	+̲	ftr.	0	0	0
	4 "	+	+ ±	+ +	+ +	+ +	+ ±	+̲	0	0	0
	overnight	+	+ ±	+ +	+ + ±	+ +	+ ±	+	ftr.	fftr.	0
Fraction D 1	½ hr.	0	0	0	ftr.	ftr.	0	0	0	0	0
	4 "	0	ftr.	±	+̲	+	±	tr.	0	0	0
	overnight	ftr.	tr.	+̲	+	+	+	±	tr.	0	0
Fraction D 3	½ hr.	0	0	0	0	0	0	0	0	0	0
	4 "	0	0	0	0	0	0	0	0	0	0
	overnight	ftr.	fftr.	fftr.	fftr.	0	0	0	0	0	0

demonstrated with various albumose fractions some of which were precipitable only to a very slight degree by the immune sera. Such tests are presented in Table II. Cross tests with albumoses from pig serum proved that the precipitation reactions as well as the inhibition effect are distinctly specific.

TABLE II.
Inhibition Tests.
To 0.2 cc. of a 1:500 dilution of 5% sheep heteroalbumose, 0.1 cc. of saline (control) or albumose solutions tested for inhibition in the concentrations indicated was added, then 2 drops of immune serum.

Concentration of albumose solutions added	Reading after	Hetero-albumose	Fraction D 1	Fraction D 3	Control
0.25%	2 hr.	tr.	0	fftr.	+±
	overnight	tr.	fftr.	±	++
0.125%	½ hr.	fftr.	0	0	+
	2 "	tr.	fftr.	tr.	+±
	overnight	±	ftr.	±	++

It may be mentioned that the inhibition phenomenon was observed with albumoses which were readily dialyzable through collodion membranes.

6957

Influence of Ovarian Grafts upon Immature Castrate Rats.

JAMES L. MANDEL. (Introduced by Robert Chambers.)
From Washington Square College, New York University.

Hohlweg and Dohrn[1] recently reported the induction of precocious sexual maturity in immature castrate female rats through the implantation of infantile ovarian tissue 3 weeks after oophorectomy. In connection with some experimental work on the anterior pituitary the writer had occasion to confirm and extend their observations.

Ten to 14 day old females, each with litter mate controls both normal and castrate, were gonadectomized and allowed to run for approximately 21 days. Two immature ovaries were then implanted intramuscularly in the experimental animals, the donor age varying from 9 to 35 days. At the time of vaginal opening the animals were sacrificed and grafts and pituitaries were prepared for histological study. The results are summarized in Table I.

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[1] Hohlweg, W., and Dohrn, M., *Klin. Wochenschr.*, 1932, **11**, 233.

TABLE I.
Effect of ovarian transplantation on time of sexual maturity.

			Time of vaginal opening		
Litter	Age at transplantation, days	Age of transplants days	Experimental days	Unoperated controls days	Castrate controls days
1	35	14	42	59	—*
2	35	35	78**	53	82, 70
3	34	22	46	61	—*
			50		
4	37	32	41	45	—*
5	35	30	41	51	76
6	36	16	46	53	92
7	34	30	42	42	67
			65**		
8	37	14	46	61	73
			47		
9	34	14	73**	53	73
10	33	24	41	56	68
			43		
11	32	9	42	64	—*
12	32	9	42	52, 54	67
13	34	18	42	57	82

* No castrate control.
** Transplant unsuccessful.

It will be seen that in every instance in which the ovarian grafts proved successful the experimental animal matured considerably earlier than its litter mate controls, as evidenced by vaginal canalization and by an oestrous smear. When the experimental animal failed to mature precociously, histological examination of the grafts revealed absence of ovarian tissue. Moreover, in unsuccessful cases, the experimental animal and the castrate controls matured at about the same time—considerably later than the unoperated control. Results show also that the experimental animals usually matured within 7-10 days, despite the fact that the age of ovaries employed for transplants varied from 9 to 35 days. In 2 instances (Litters 4 and 7) the normal animals matured too early to render results of any significance.

It appears probable from these data that the castration pituitary stores sex hormone which is liberated under the influence of the transplanted ovaries, causing the latter to mature precociously. It has been adequately demonstrated (Engle,[2] Evans and Simpson[3]) that the castration pituitary in the adult rat is more potent physiologically than the normal gland.

Despite the fact, however, that sexual maturity characterized the experimental animals the anterior pituitaries displayed a marked castrate condition. Thus the anomalous situation presents itself of a precociously matured rat, in oestrus at time of sacrifice, possessing what appears to be a typical castration pituitary. The most obvious interpretation appears to be that, although the implanted gonads effected a release of secretion from the pituitary, the time interval before sacrifice was not sufficient for the gonads to induce correction of the castrate picture. None of the grafts were allowed to remain over 16 days. Thus the time required for a complete return to normal would appear to lie between 16 and 30 days, Haterius and Nelson[4] having reported a complete correction to have occurred at the latter figure. This point is now being studied in animals carrying grafts over a longer period.

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[2] Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

[3] Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 371.

Successful grafts were characterized by the presence of enormous cystic follicles, the walls of which were becoming markedly luteinized. In some instances very large luteal masses were present, comparing very favorably with the histological picture of ovaries from animals subjected to anterior lobe administration.

6958

Experimental Poliomyelitis: Evidence of Early Involvement of Central Nervous System.

S. D. KRAMER AND G. C. PARKER. (Introduced by A. J. Goldforb.)

From the Laboratories of the Infantile Paralysis Commission and Department of Neuropathology, the Long Island College of Medicine, Brooklyn, N. Y.

The epidemic of poliomyelitis in New York and New England in 1931 presented an opportunity for controlled studies[1, 2] to determine the value of convalescent and other immune serums, administered in the preparalytic stage of the disease. In these reports the authors were unable to present statistical evidence that such serums were of value, either in reducing the death rate or in preventing paralysis. Since experience with virus diseases indicates that little is to be expected from the use of immune serums, once the disease has become established in the organism, it becomes important to determine the extent of involvement, particularly of the central nervous system, in the preparalytic stage of poliomyelitis.

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[4] Haterius, H. O., and Nelson, W. O., *J. Exp. Zool.*, 1932, **61**, 175.

[1] Kramer, Aycock, Solomon and Thenaby, *N. Eng. G. of Med.*, 1932, **206**, 432.

[2] Park, William H., *Transactions Assn. Physicians*, 1932, **47**, 123.

In this study, an effort was made to determine the early pathology in the experimental disease, following intranasal instillations of the virus. The close analogy between experimental poliomyelitis and the human disease should make any findings in the former significant in their application to the latter.

We have recently succeeded in obtaining a nasal strain of virus* which has successfully infected every animal. This mode of infecting the experimental animal is the probable mode of infection in man, and eliminates the trauma incident to intracerebral inoculation.

Method. Six *Macacus rhesus* were simultaneously inoculated intranasally on 3 successive days with a 10% centrifuged suspension of cord in distilled water. 1 cc. of the suspension was instilled into each nostril. Temperatures were taken twice daily and the animals observed for evidence of illness. They were sacrificed in the following order: The first animal 24 hours after the third instillation, and one animal each day thereafter until the 6 had been killed. The animals were killed with chloroform and autopsies performed immediately. The brains and cords were removed *in toto* and fragments from different levels and areas fixed in 10% neutral formalin, Zenker's solution and 95% alcohol. Sections were also taken from the abdominal and thoracic viscera and from peripheral nerves. The present report gives the results of histologic findings in the brains and cords, using hemotoxylin eosin and Nissl stains. One additional animal that did not receive the intranasal instillations, was sacrificed to serve as a control. This animal was not entirely normal inasmuch as it was suffering from a prolapsed rectum.

The first two animals sacrificed had no elevation of temperature and showed no evidence of illness. The third animal showed no signs of illness, but had an elevation of temperature for several hours before it was killed. The fourth had a temperature for 24 hours, a mild tremor which became evident only after severe exertion, and no evidence of paralysis. The fifth animal presented all the classical findings of the experimental infection, elevation of temperature, tremor, ruffled fur, staccato cry and ataxic gait, but no paralysis. The animal could climb but preferred to crouch quietly. The sixth and last animal presented all the findings of the fifth, and in addition, had flaccid paralysis of the left shoulder and arm.

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* This virus was obtained through the kindness of Mr. M. Schaeffer of the Department of Bacteriology, New York University.

TABLE I.

Monkey No.	36	34	31	33	35	32	64
Virus (Mixed Nasal Strain)	1 cc. in each nostril	1 cc. in each nostril	1 cc. in each nostril	1 cc. in each nostril	1 cc. in each nostril	1 cc. in each nostril	None
Dates Inoculated	2/28/33 3/1/33 3/2/33	2/28/33 3/1/33 3/2/33	2/28/33 3/1/33 3/2/33	2/28/33 3/1/33 3/2/33	2/28/33 3/1/33 3/2/33	2/28/33 3/1/33 3/2/33	— — —
Date Sacrificed	3/3/33	3/4/33	3/5/33	3/6/33	3/7/33	3/8/33	5/3/33
Clinical Signs	No signs of illness No elevation of temperature	No evidence of illness No elevation of temperature	Temperature elevation on day sacrificed No other sign of illness	Elevation of temperature Slight tremor Otherwise negative	Elevation of temperature Tremor Ruffled fur Staccato cry Ataxic gait No paralysis	Elevation of temperature Tremor Ruffled fur Staccato cry Paralysis of left shoulder and arm	Prolapse of rectum Somewhat less active than normal animal
Outstanding[1] Positive Pathologic Findings	Infiltration[2] of gray matter and dorsal root ganglia Pial edema Edema of cord Congestion Hemorrhage Ganglion cells: Chromatolysis Neuronophagia (occasional)	Infiltration[2] Edema of cord Congestion Hemorrhage Ganglion cells: Chromatolysis Neuronophagia (occasional)	Infiltration[2] Edema of cord Pial edema and hemorrhage Congestion Hemorrhage Ganglion cells: Chromatolysis Neuronophagia	Infiltration[2] Edema of cord Perivascular infiltration Hemorrhage into pia and cortex of brain and gray matter of cord Ganglion cells: Chromatolysis Neuronophagia	Marked infiltration[2] Marked edema of cord Perivascular infiltration Congestion Hemorrhage Ganglion cells: Chromatolysis Neuronophagia	Infiltration[2] Marked edema of cord Perivascular infiltration Congestion Hemorrhage Ganglion cells: Chromatolysis Neuronophagia Marked reduction in numbers	Sl. infiltration with lymphocytes Sl. edema of cord No congestion No hemorrhage No meningeal involvement Ganglion cells: showed no changes that could be considered significant

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[1] Although not clearly indicated in the table, the lesions were progressive, being more marked and extensive in successive monkeys, especially in the last three.

[2] This refers to a diffuse cellular infiltration by lymphocytes, plasma and glial cells and some polymorphonuclear leucocytes.

Sections of the brains and cords showed progressive involvement of central nervous system in all of the animals (Table I).

These sections therefore presented evidence of involvement of the central nervous system as early as the third day following the first intranasal instillation, at least 2 days before the appearance of temperature, and 5 days before the appearance of paralysis.

These results not only cast doubt on the therapeutic value of immune serums in preparalytic poliomyelitis, but also help to explain the failure of immune serums to modify the experimental disease; and by analogy offer a similar explanation of the indifferent results of the controlled series of human cases in 1931.

The authors wish to express their thanks to Dr. William H. Park, Director of the Bureau of Laboratories, New York City, for his help and cooperation.

6959

Meteorological Influences on Blood pH and Cholesterol.

WM. F. PETERSEN.

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

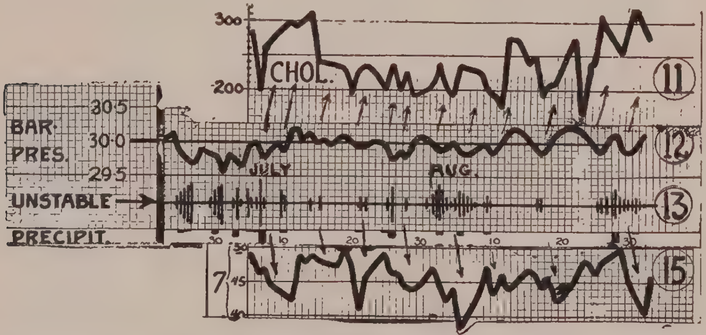
Among the blood chemical levels that are distinctly altered with each passing cyclonic wave are the blood pH and the cholesterol. The anoxemic stimulus to which I have previously referred is almost invariably followed by striking increase in the cholesterol level, an increase that is much greater than can be attributed to an alteration in the concentration of the serum.

When determinations are made day by day in the normal individual the fluctuations in the level of cholesterol will show a curve with a range of more than 100 mg., in the unstable person the range may be more than 300 mg.

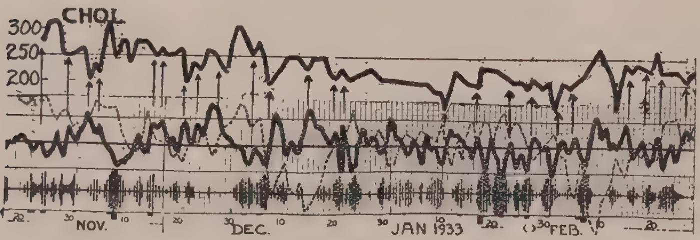
The anoxemic stimulus results in greater liberation of acid metabolites and as might be anticipated, each cyclone is associated with an acidity which follows in its wake, the degree and duration of which is, of course, a purely individual matter. It depends on the constitutional type, on the buffering, on the season, etc. In unstable persons the fluctuations may be quite marked. While the change in the pH may be abrupt, day by day determinations reveal a distinct sequence of increasing and decreasing levels. When meteorological stimuli are either very pronounced or frequent, summation effects are readily evident. In the leptosome the buffer mechanism is more apt to be depleted as a result of a series of meteorological adjustments; the pyknic appears to be much better buffered. Two small graphs are appended to illustrate these chemical alterations.

In the first one, curve 11 represents the cholesterol levels, curve 12 is the barograph, curve 13 is the graph of unstable weather with the date line below, curve 15 is the pH level. Arrows have been carried from barometric depressions to cholesterol increase. They have also been carried down from points of unstable weather (barometric lows) to the corresponding low pH levels that follow. This graph is that of a perfectly normal individual of pyknic habitus and covers a period of July and August, 1932.

In the second graph the cholesterol curve has been superimposed over the meteorograph (the barometric pressure is indicated by



Graph 1.
Effect of meteorological alterations on blood pH and Cholesterol. Normal individual, pyknic habitus.



Graph 2.
Blood cholesterol and the relation to the meteorological alterations. Psychopathic individual.

the heavy black line—the temperature is indicated by the dotted line) for a period extending from October to March and is taken from determinations made on a psychopathic patient. Arrows have been carried from barometric lows to corresponding cholesterol lows merely for purposes of orientation. In general the curves form a mirror image but individual variations occur from case to case.

Conclusions. Day by day determinations made in a large series of normal persons and in patients reveal a distinct rhythm of increasing and decreasing pH as well as of cholesterol. Meteorological associations indicate that each cyclonic cycle is associated with a relative increase in hydrogen-ion concentration and an increase in cholesterol.

6960

Complete Recovery of Gonadotropic Substances from Urine of Women.

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From the Department of Medicine, University of Wisconsin.

For assay of urine concentration of gonadotropic substances in normal or non-pregnant women it is necessary to prepare concentrated extracts which are not toxic to immature female rats. Complete recovery of active material is highly desirable. Tests on 6 published extraction methods have failed to yield satisfactory total recovery when applied to urine from pregnant women. The various steps in some of these processes have been studied, and sources of loss have been identified.

Decrease in potency from concentrating urine by low temperature evaporation is probably not so much a temperature effect as due to the adsorption on the urinary solids. Washing these solids with 33% acetone at pH 5 yielded significant amounts for each of 5 washings.

Acetone precipitations of gonadotropic material from urine are incomplete unless the acetone is 95% (20 volumes) at room temperature or 16 volumes at 38°C. The acetone precipitation alone does not remove all toxic materials.

Urine acidified to pH 5 with glacial acetic acid will allow of complete adsorption of gonadotropic substances by Lloyd's reagent or norit. Permutit was less active. Elution from norit was, however, incomplete with a number of reagents. From Lloyd's reagent partial recovery was possible with ammonia-free distilled water, 33% acetone, 50% acetone, or 0.1 N sodium hydroxide. Complete recovery was found with 50% aqueous pyridine, or alkalinized acetone (50%). Better yields were obtained with equal parts of C.P. acetone and 0.2 N sodium hydroxide than with two parts of alkali to one of acetone.

The following extraction method is proposed to secure non-toxic extracts from pregnancy urine without appreciable loss of gonadotropic potency.

1. Acidify urine to pH 5 with glacial acetic acid, filter, and shake for 2 hours with 20 gm. of Lloyd's reagent per liter. Readjust to pH 5 and filter by suction. Discard urine, dry and return the adsorbent to the flask.

2. a. *Pyridine method.* Add 50% pyridine in amount equal to the volume of urine, shake continuously for 8 hours, and remove the solvent by filtration or centrifugation. Repeat the extraction with a second equal portion of 50% pyridine for 8 hours. Wash the residue with small amounts of 33% acetone, adding the washings to the extracts.

b. *Alkaline acetone method.* Mix equal parts of C.P. acetone and 0.2 N sodium hydroxide, and extract as with pyridine, except that extractions are limited to 4 hours each. (Six hours causes some loss.) Wash with 33% acetone.

3. As soon as extracts are removed from the Lloyd's reagent pour them into 16 volumes of acetone at 38°C. Keep the material at about 8°C. until the precipitate has subsided. Siphon off the liquid and place the container with the precipitate in the breeze of a fan to remove the rest of the acetone. Take up the dried precipitate in water or saline for injection.

Extracts prepared by this method have been assayed for potency by subcutaneous injection of amounts equivalent to the amount of untreated urine demonstrated to contain the minimal effective dose of gonadotropic substance. The minimal effective dose of untreated urine is considered to be the least amount of that urine which injected in 5 daily portions produces in at least one of 3 rats, 24 days of age at the beginning of the injections, a 100% increase in ovary weight and one or more corpora lutea at necropsy on the sixth day. The fact that approximately duplicate ovarian effects are produced by the minimal effective dose of the whole urine and by an equivalent amount of the extract is offered as evidence that satisfactory total recovery of active material is obtained by this method. Injection of 100 cc. urine equivalents of the extracts results in no toxic effects. The extracts are apparently free from follicular hormone since they are without effect on the uteri of immature castrate female rats of the same age.

6961

Basophilic Activation of Neurohypophysis and its Bearing on Certain Diseases Characterized by Hypertension.*

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From Harvard Medical School, Boston.

Of the 3 cellular elements composing the pituitary body, the basophilic cells are normally fewest in number and their function least well understood. A peculiar disease, apparently due to a functionally active adenoma of these elements, has recently been described[1] and a further example of this disorder has been thoroughly examined after death.[2] In this case, the unenlarged pituitary body in addition to the basophilic adenoma of the pars anterior shows a heavy invasion of the posterior lobe by basophilic elements from the pars intermedia.

One of the characteristic symptoms of this disease is a high blood-pressure, and the posterior lobe of glands from such fatal cases of eclampsia and of essential hypertension as I have since had the opportunity to study, have shown the same type of massive basophilic infiltration.

The active hormone of the posterior lobe is unquestionably derived from the pars intermedia whose fully ripened elements are indistinguishable from the basophils of the pars anterior. The ripened cytoplasm of the inwandering cellular elements becomes transformed into the secretory product (the hyaline bodies of Herring) which can be traced in favorably fixed tissues up the pituitary stalk to the region of the tuberal nuclei. The open meshwork of the tuberal tissue and the invariably broken-up appearance of the cuticular ependyma, lining the infundibular tip of the third ventricle, strongly suggest the passage of a secretory product into the ventricular cavity.

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* Abstract of report before the National Academy of Science, April 25, 1933.

[1] *Bull. Johns Hopkins Hosp.*, 1932, **50**, 137.

[2] *Arch. Int. Med.*, 1933, **51**, 487.

It is well known that from the supraoptic and tuberal nuclei an abundance of unmyelinated fibers pass down the pituitary stalk to arborize richly in pars nervosa and pars intermedia. Karplus and Peczenik[3] have recently shown that stimulation of the tuber causes a posterior-pituitary substance to appear in the cerebrospinal fluid.

Under normal circumstances, histological evidences of posterior lobe activity are inconspicuous, and the massive basophilic invasion described above is consequently interpreted as nothing more than an exaggeration of the normal secretory process. It provides a reasonable explanation of certain of the striking symptoms which accompany not only basophilic adenomas but also the symptomatically related states of eclampsia and essential hypertension.

Hofbauer[4] was apparently the first to suggest that the toxemias of pregnancy might be due to posterior-pituitary activation. This hypothesis was strongly supported when Anselmino and his coworkers[5] demonstrated an excess of a posterior-pituitary-like substance (both antidiuretic and haemodynamic) in the blood of patients with hypertensive forms of eclampsia.

Should the present observations regarding posterior lobe basophilia be confirmed, not only eclampsia but other hypertensive disorders, for which there has heretofore been no wholly satisfactory pathological explanation, may prove to be states which are closely related.

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[3] *Pflüger's Arch.*, 1933, **232**, 402.

[4] *Zentralbl. f. Gynäk.*, 1918, **42**, 745.

[5] *Edinburgh M. J.*, 1932, **39**, 376.

6962

High Carbohydrate and High Fat Diets.

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GERTRUDE THOMAS.

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During the course of a study of glucose tolerance in relatives of diabetics[1] we became interested in determining the effect of various diets on glucose tolerance, and on some of the constituents of the blood.

One of us served as subject. Arrangements were made with the University Hospital for weighed diets of known caloric value. The diet for the first 7 days, Period 1 (March 6 to 12 inclusive), was a balanced one; that for the second 7 days, Period 2 (March 13 to 19 inclusive), was high in carbohydrate; during this period, carbohydrate constituted more than 90% of the total caloric intake. The balanced diet was used for the third 7 day period, Period 3 (March 20 to 26 inclusive). The diet for Period 4 (March 27 to April 2 inclusive), was high in fat. The ketogenic-antiketogenic ratio was 2:1 for the first 3 days, 2.5:1 for the fourth day, and 3.0 to 1 for the fifth, sixth and seventh days. Acetonuria was present from the fourth day on. The diet for Period 5 (April 3 to 6 inclusive) was balanced. The total caloric intake varied somewhat during the experimental period, according to the subject's wishes.

TABLE I.

Date		Carbohydrate	Protein	Fat	Total
			Calories		
Period I	Min.	852.0	183.6	489.6	1527.1
3/6–3/12	Max.	1016.0	216.4	516.6	1749.0
	Aver.	922.3	197.4	510.8	1630.4
Period II	Min.	1120.0	71.2	14.4	1205.6
3/13–3/19	Max.	1143.9	79.1	14.9	1237.9
	Aver.	1130.3	77.2	14.6	1222.0
Period III	Min.	665.0	158.8	387.3	1242.8
3/20–3/26	Max.	823.7	218.7	514.6	1554.0
	Aver.	739.7	189.2	434.2	1364.7
Period IV	Min.	62.7	97.6	1164.8	1552.0
3/27–4/2	Max.	253.0	164.1	1401.5	1630.0
	Aver.	117.5	148.8	1326.6	1601.3
Period V	Min.	820.4	211.5	497.5	1536.0
4/2–4/6	Max.	954.4	241.9	563.6	1778.0
	Aver.	888.2	229.2	539.5	1664.2

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[1] Greisheimer, E. M., and Goldsworthy, E., *Am. J. Physiol.*, in press.

Table I presents the total caloric intake, and the distribution among the various foodstuffs for each period.

Glucose tolerance was tested at intervals during the experimental period; the results are presented in Table II. One gram of glucose for each kilo of body weight was ingested. It will be noted that a diabetic response was secured after the ketogenic diet. The subject's usual type of response appeared shortly after cessation of the ketogenic diet.

TABLE II.

Date		Fasting	½ hr.	1 hr.	2 hr.	3 hr.
3/13/33	After 7 days on balanced	81.3	106.5	120.9	94.8	63.4
3/20	After 7 days on high carbohydrate	86.2	115.3	115.6	94.4	70.1
3/27	After balanced diet	82.0	117.9	120.5	103.7	81.8
4/3*	After ketogenic diet	66.9	115.8	154.9	201.5	134.7
4/4	One day after cessation of ketogenic diet	82.2	127.7	154.2	132.3	102.3
4/7	4 days after cessation of ketogenic diet	81.8	110.0	109.5	108.6	89.5

*The urine showed acetone and sugar.

Twelve determinations of inorganic phosphate, calcium, total non-protein nitrogen, urea nitrogen, blood sugar, hemoglobin (Newcomer method), red count, and white count were made between March 8 and April 7, 1933. Marked variations occurred in total non-protein nitrogen (29.5 to 47.7 mg.), urea nitrogen (6.41 to 18.67 mg.), and sugar (64.1 to 86.4 mg.). It should be kept in mind that these constituents are influenced by diet.

The inorganic phosphate varied slightly during the study, averaging 3.1, 3.3, 3.4, 2.8 and 3.9 for the 5 periods. None of these values are beyond the limits of normal, and are not of clinical importance.

The serum calcium averages were 10.39, 10.12, 10.23, 10.61 and 10.42 for the 5 periods; the differences are not important clinically.

The urea nitrogen averages vary greatly; the values were 8.34, 9.49, 17.69, 15.90 and 18.09 for the 5 periods. With the exception of the first period, the urea nitrogen roughly parallels the protein intake.

The total non-protein nitrogen averages were 39.9, 34.4, 41.1, 31.8, and 38.1 for the 5 periods. The lowest value was found on the high fat diet even though the protein intake was not lowest at this time. The other constituents of the diet influence the total non-protein nitrogen.

The fasting blood sugar is remarkably constant on a balanced diet.

It is altered but little by an increase in the intake of carbohydrate. The values found in the different periods were 82.2, 83.3, 81.3, 65.5, and 81.8. It will be noted that the fasting sugar drops to a fairly low level on the high fat diet. It seems that a decrease in carbohydrate intake has more effect than an increase.

The average weight, temperature on arising, temperature on retiring, fluid intake, and fluid output for each period are given in Table III.

TABLE III.

Period	Wt. lb.	Temperature on arising	Temperature on retiring	Fluid intake	Fluid output
I	117	98.3	98.2	1086	797
II	115	97.9	97.3	614	619
III	113	98.6	98.1	843	614
IV	111	98.6	98.6	1046	726
V	111	98.5	98.6	1018	625

There was some weight loss during the period of study. The morning and evening temperatures showed no remarkable variations from normal. Since the only fluid output measured was the urine, no conclusions of value can be drawn from the relation between intake and output on the various diets.

Summary. The glucose tolerance was not significantly altered by a very high carbohydrate intake, over a period of 7 days. After 7 days on a high fat diet, the response to the glucose tolerance test was typical of diabetes mellitus; the tolerance rapidly returned to the usual type, after return to the balanced diet. Since the diet can so markedly influence the tolerance, this should be kept in mind in glucose tolerance studies. The urea nitrogen and total non-protein nitrogen vary markedly with different dietary conditions. This fact is of clinical importance. The fasting blood sugar is markedly influenced by a diet low in carbohydrate. A high intake of carbohydrate seemed without effect on the fasting blood sugar. It was planned to make more extensive studies, but the subject developed undulant fever, and the experimental work was necessarily postponed.

6963

Ether Barbiturate Anesthesia.

J. T. HALSEY and C. F. LACEY.

From the Department of Pharmacology, Tulane University School of Medicine.

That barbiturates as well as other non-volative anesthetics (hypnotics) act additively with ethylene or nitrous oxide has been conclusively shown by several investigators[1, 2, 3, 4] as also by clinical experience. That this also holds good with ether seemed to have been shown by clinical observations. Consequently considerable surprise and doubt arose when Calderone[5] reported that in dogs he had been unable to find any evidence that there was any additive effect of barbiturates when given with ether. This communication brings a brief report of the results obtained by us in further investigations of this problem.

Methods. Dogs were used throughout. Under light ether anesthesia, carotid and tracheal cannulae were inserted and the tracheal cannula connected with a leak proof system containing soda-lime into which oxygen and ether could be introduced as needed. The ether concentration in the system was then slowly increased until various degrees of anesthesia were obtained. By frequent sampling, the ether, carbon dioxide, and oxygen concentrations were determined and either increased, diminished, or kept constant as was desired. In 5 experiments intravenous injections of 10 mg./K Nembutal (20% of M.L.D.) and in one of 20 mg./K of Dial-Ciba (20% of M.L.D.) without exception produced so unmistakable an increase in the depth of the anesthesia that no other conclusion can be drawn than that these two barbiturates act additively with ether.

Data from a typical observation follow: Dog C from 9:41 to 10:54 respired a mixture in which the ether concentration was gradually increased from 2.7 to 3.1%, corneal and toe reflexes (motor response to crushing of toe) and muscle tone not being abolished. A concentration of 3.7% of ether respired from 11:00 to 11:21 induced and maintained almost complete surgical anesthesia, muscular relaxation being complete although corneal and toe reflexes were faintly positive. When ether percentage was reduced to 2.6 at

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[1] Stormont, Lampe, and Barlow, *J. Pharmacol. and Exp. Ther.*, 1930, **39**, 165.

[2] Barlow, Duncan and Gledhill, *J. Pharmacol. and Exp. Ther.*, 1931, **41**, 367.

[3] Kleindorfer and Halsey, *J. Pharmacol. and Exp. Ther.*, 1931, **43**, 449.

[4] Swanson, E. E., *J. Pharmacol. and Exp. Ther.*, 1932, **46**, 389.

[5] Calderone, Read at Anesthetic Congress, N. Y., Oct., 1932.

12:03, both reflexes were active and muscular relaxation was incomplete. By 12:46 with ether percentage at 2.1, corneal and toe reflexes were very active and there was little or no muscular relaxation. Intravenous injection 12:48 to 1:03 of 20 mg./K of Dial-Ciba was followed within two minutes by a state of profound anesthesia with complete muscular relaxation, disappearance of the corneal reflex and almost complete abolition of motor response to violent crushing of the toe. This condition remained unchanged for 1 hour, the ether being kept at 2.0 to 2.1%. At 2:25, with ether reduced to 1.6%, muscle relaxation became incomplete and corneal and toe reflexes became active again. With the shorter acting Nembutal, similar results were obtained except that after 15 to 30 minutes, the anesthesia became progressively less intense. Numerous observations on the effects of Dial-Ciba and Nembutal in the dosage used in these experiments have shown that by themselves they do not appreciably affect muscular relaxation or the activity of the corneal or toe reflexes.

The effect of morphine on ether anesthesia will be reported at a later date.

6964

Observations on Coronary Occlusion.*

F. A. SUTHERLAND, DAVID DIAL AND B. R. HARRIS.
(Introduced by Raymond Hussey.)

From the Departments of Pathology and Surgery, Yale University School of Medicine.

A series of experiments were made to correlate the anatomic, electrocardiographic and myocardial surface temperature changes following ligation of the anterior descending branch of the left coronary artery. The effect of stellate ganglionectomy on these changes was also studied. Seventeen dogs were used.

Under amytal anesthesia, with respirations maintained by positive pressure the pericardium was exposed by cutting a window through the anterior portion of the left 5th and 6th ribs. The pericardium was opened and sutured to the adjacent pleura. The stellate ganglion was exposed by an anterior axillary approach. The anterior descending branch of the left coronary artery was ligated 1 or 2 cm. from its bifurcation. No attempt was made to separate the artery from its vein. Both vessels were ligated by means of a single suture. Thermocouples were sutured onto the surface of the myocardium, one being placed in the region supplied by the ligated artery, the other in a region with an independent blood supply. Thermal changes over each area were recorded on a Leeds and Northrup Potentiometer System. Simultaneous records of room and rectal temperatures were obtained. Immediately following coronary artery ligation transient cyanosis and bulging of the surface area supplied by the ligated vessels was noted. In every instance the surface temperature of the ischemic area after ligation fell from 2-7°F., and persisted throughout the period of observation.

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* This investigation is supported by funds from the Josiah Macy, Jr., Foundation.

Ventricular fibrillation, which was noted in 7 dogs, appeared under the following conditions: (1) In 3 dogs immediately following ligation of the artery; (2) In one dog during suture of the pleura and pericardium; (3) In 2 dogs following release of a vessel which had been ligated for 1 hour and 40 minutes and 3 hours and 40 minutes respectively; and (4) In one dog by manipulation of the heart by a suture needle 96 hours after ligation of the artery.

No changes in the myocardial surface temperature were noted following bilateral stellate ganglionectomy, faradic stimulation of the stellate ganglia, bilateral vagotomy, or following subcutaneous or intracardiac injection of adrenalin.

Histologic study of the heart from areas supplied by the ligated vessels showed no demonstrable characteristic changes in animals surviving for less than 10 hours, with one exception. In this case small focal areas of accumulation of fat droplets in the myocardial fibers was noted. In all those experiments in which the heart was exposed to the air for a period longer than 2 hours a fibrino cellular exudate appeared on the pericardial surface. In the epicardium beneath this exudate polymorphonuclear cellular infiltration and edema was present. In one case a small early mural thrombus was observed in the ligated artery. Three dogs following coronary ligation were allowed to survive 24, 48 and 96 hours, respectively. The myocardium of the animal surviving 24 hours showed intracellular fat droplets. The dogs surviving 48 and 96 hours showed areas of frank necrosis of the myocardium, some polymorphonuclear cell infiltration and hemorrhage. These changes are consistent with those reported by Karsner and Dwyer.[1] In addition to the myocardial changes in these animals there was a marked pericarditis, involving also some of the external muscle fibers, and showing some fibroblastic proliferation.

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[1] Karsner and Dwyer, *J. Med. Research*, 1916, **34**, 21.

Simultaneous electrocardiographic studies confirmed the presence of ventricular fibrillation as noted above. Successive changes in the R T interval and the T-waves usually associated with coronary occlusion were noted in the longer experiments. In 2 dogs simple opening of the pericardium produced changes in the T-waves similar to those described as due to coronary occlusion. No uniform electrocardiographic changes were noted in animals surviving less than 10 hours.

6965

Absorption Properties of the Intima of the Carotid Artery.*

ELIZABETH M. RAMSEY AND LOUIS K. ALPERT.
(Introduced by Raymond Hussey.)

From the Department of Pathology, Yale University School of Medicine.

In the course of a series of studies on the structure and function of vessel walls it was determined to investigate the absorption properties of the intima by injecting a diffusible substance into the lumen of a closed vessel. Dogs were used as experimental animals and the common carotid artery was the vessel chosen. For the diffusible substance phenolsulphonphthalein was selected because it has no effect upon tissues and, if absorbed, can be readily detected in the urine in small amounts. The carotid sheath was exposed and the artery doubly ligated without opening the sheath. The ligatures were placed as far apart as possible to allow a maximum of absorption surface, but great care was exercised that no branches should be included in the stretch between the ligatures. The dye was injected through a cannula at a constant pressure equivalent to the carotid pressure (determined for each animal on the contralateral vessel). At the termination of the experiment the phenolsulphonphthalein was evacuated and 1% trypan blue was run through the same system at the same pressure to exclude the possibility of leakage in the system. In a series of 7 animals the dye appeared in the urine in all instances. The appearance time varied between 48 and 141 minutes, whereas the appearance time was 4 to 8 minutes in several

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*This investigation is supported by funds from the Josiah Macy, Jr., Foundation.

control dogs to whom 1 cc. of the dye was administered by the usual intravenous route. No obvious explanation of the delay in excretion was apparent, but, as might be expected, analysis of the data shows that the amount of dye absorbed is a function of the length of vessel included between the ligatures.

6966

Response of Tissue of the Intima to Injurious Agents.*

ELIZABETH M. RAMSEY AND LOUIS K. ALPERT.
(Introduced by Raymond Hussey.)

From the Department of Pathology, Yale University School of Medicine.

A series of experiments was designed to investigate the nature of the reaction exhibited by the intima when brought in contact with an injurious agent. Eleven arteries in 8 dogs were subjected to the experiment. The common carotid artery was isolated and doubly ligated according to the procedure previously outlined.[1] Various strengths of turpentine, ranging from 15% in mineral oil to the crude substance, and 30% to 75% croton oil in mineral oil were injected into the lumen of the vessel by syringe or through a cannula. The injurious agent was allowed to remain in contact with the intima for periods varying from 2 minutes to 48 hours and then the portion of the artery between the ligatures was removed for microscopic examination. As controls, to determine how much reaction was caused by the operative procedure alone, histological preparations were made of the arteries which were used in the phenolsulphonphthalein experiments.[1] In these the endothelium was uniformly intact and well stained. The muscular, elastic and fibrous tissue elements of the media showed no changes. There was no necrosis or hemorrhage in any of the coats and no exudate at any point in the vessel wall proper. Surrounding some of the vessels lying in the connective tissue outside the adventitia were small collections of polymorphonuclear leucocytes, but in no instance were these seen infiltrating the adjacent tissue. Microscopic examination of the arteries exposed to an injurious agent showed destruction of the endothelium lining the vessel. In the instances where the weaker dilutions of turpentine were employed, poorly stained remnants of this layer could be seen, but with the other injurious agents it was completely absent. When 50% and 75% croton oil and higher dilutions of turpentine were used the nuclei of the muscle cells in the inner third of the media were pyknotic and a slight fraying of the elastic fibers in the media, including the internal elastic lamella, was present. This latter change appeared to be of a corrosive type. There was no exudate in the intima or the tissues immediately beneath the internal elastic lamella, but as the adventitia was approached a sparse scattering of small round cells and occasional polymorphonuclear leucocytes was observed in 2 instances. Small clumps of polymorphonuclear leucocytes were seen surrounding the vessels in the periadventitial connective tissue in all the sections, but the number of cells did not exceed that observed in the controls. From these observations it must be deduced that the tissues of blood vessel walls differ strikingly from other tissues in the body with respect to their response to the injurious agents employed.

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*This investigation is supported by funds from the Josiah Macy, Jr., Foundation.

[1] Ramsey, E. M., and Alpert, L. K., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1432.

6967

Possible Relationship Between Cod Liver Oil and Muscular Degeneration of Herbivora Fed Synthetic Diets.*

L. L. MADSEN, C. M. MC CAY AND L. A. MAYNARD.

From the Laboratory of Animal Nutrition, Cornell University, Ithaca, N. Y.

We have made numerous attempts to formulate a satisfactory synthetic diet for herbivorous animals. In a previous communication,[1] in which partial success with an adult goat was recorded, it was reported that the synthetic diet caused paralysis in rabbits with a histological picture of muscle degeneration. These studies have been continued with goats, rabbits and guinea pigs, using diets as follows:

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* Supported by a grant from the Heckscher Research Fund.

[1] Woodward, J. C., and McCay, C. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 241.

Goats		Rabbits and Guinea Pigs
20	Regenerated Cellulose	20
26	Corn Starch	40
20	Casein	15
15	Sucrose	10
7	Yeast	5
6	Lard	4
6	Salt Mixture	4
12 cc. per week	Cod Liver Oil	2
	Tomato or Orange Juice	Daily

Six kids were transferred to the diet directly after weaning. They consumed it readily and grew at a normal rate for 2½ to 3 months, when the growth rate declined but all animals continued to eat well. The first death occurred after 133 days on the diet and 4 others died during the following 2 months. The remaining animal lived until the 315th day. All animals died suddenly without obvious symptoms.

The characteristic finding on autopsy was the evidence of failing circulation, consisting of intermuscular edema of the legs, increased fluid in body cavities, and congestion and edema of the lungs. There was frequently a severe hemorrhagic enteritis, often associated with large amounts of blood stained fluid in the lumen of the small intestine. Fatty degeneration of the liver and kidneys was also a frequent finding. Although no case of stiffness, such as is found regularly in the rabbits, was observed, gross muscle lesions were found in several of the goats. On histological examination muscle lesions were found in all cases. The skeletal muscle lesions were similar to hyaline, waxy or Zenker's degenerative changes. There was also a proliferation of fibroplastic tissue which tended to replace the degenerating muscle.

The heart muscle was also involved in all animals. Gross alterations were often evident and consisted of dilations especially of the right ventricle. In some cases the heart was entirely normal in shape but the myocardium was usually paler than normal suggesting degeneration and fibrosis. On section, several local well defined grayish areas were found. On histological examination hyaline and granular degeneration changes of the heart muscle fibers were seen. In some areas the fibers were necrotic and in process of absorption. These changes were accompanied by an increase in connective tissue which often completely replaced portions of the degenerated muscle.

In continuing the studies with rabbits, the degenerative changes in the leg muscles were again found. They were similar to those seen in the goats but more extensive. The replacement of muscle by connective tissue is no doubt one of the chief causes of muscle paralysis or stiffness. Many of the rabbit hearts have shown marked gross alterations, often dilated, the muscle very flabby, the ventricles stretched and reduced in thickness. The changes usually appeared first and were more marked in the right ventricle. On histological examination hyaline changes and vacuolar degeneration of the muscle fibers were present. Hemorrhage in the heart muscle was found in a few cases. Fibroplastic changes were not as marked as in the goat hearts. However, the goat lesions were probably of longer standing.

When guinea pigs were fed the synthetic diet the results were similar to those obtained with the rabbits. Similar lesions were found in both the skeletal and heart muscles. In all the species the skeletal muscle changes resemble very closely those reported by Goettsch and Pappenheimer.[2] However, these investigators have not found any changes in the heart.

Dr. Olafson, of the Department of Pathology and Bacteriology, who first found the histological changes in the goat hearts, called our attention to the similarity of lesions to those reported by Agduhr[3] in rabbits and other species receiving large doses of cod liver oil, and suggested that this might be the causative agent in our results. Agduhr stressed the responsibility of cod liver oil in causing heart lesions but little attention has been given to his work. We, therefore studied the cod liver factor in relation to our results.

A group of 10 rabbits were placed on the synthetic diet, omitting the cod liver oil and without any added source of vitamins A or D. The fat previously supplied by the oil was furnished as lard. These rabbits made larger gains and lived longer than rabbits receiving 2 to 3% of cod liver oil in their synthetic ration, which usually die between the 30th and 40th day. By the 43rd day 2 of the rabbits on the synthetic ration without cod liver oil had died showing symptoms of vitamin A deficiency. The remaining 8 rabbits, which were starting to lose weight, were divided into 2 groups of 4 each. Group 1 received a supplement of 3% of cod liver oil and Group 2 was given a vitamin AD concentrate plus cottonseed oil to make up the fat content. All of the animals receiving the cod liver oil became stiff and died in from 11 to 19 days after the oil was introduced. There were no symptoms of A deficiency. Two of the animals placed on the concentrate refused to eat and died of A deficiency. The other 2, after a period of further weight loss, recovered and started to gain. One died after 80 days. It was not typically stiff but the heart was found flabby and dilated. The second animal is still alive and apparently normal after 270 days.

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[2] Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

[3] Agduhr, E., *Acta Paediatrica*, 1926, **5**, 319.

Another group of 4 rabbits was given the concentrate from the start. Two died at 36 days. They were not stiff but in both cases autopsy showed the lungs congested and the right ventricle slightly dilated. The other 2 survived for 155 days and 197 days respectively. The one surviving the longest was stiff for 147 days. Both showed the skeletal and heart muscle lesions on autopsy.

To study the problem further, 40 guinea pigs were divided into 4 groups as follows: (I) Basal synthetic diet with medicinal cod liver oil; (II) Basal synthetic diet with animal cod liver oil; (III) Basal synthetic diet with AD concentrate; (IV) Stock diet of alfalfa, grain and cabbage with cod liver oil.

In the previous experiment an animal grade of cod liver oil was used. Thus groups were included to ascertain whether a more refined product would give different results. The oils were fed separately as 0.5 cc. per animal per day. Group III received an equal amount of cottonseed oil containing the concentrate. The vitamin B and C sources were also fed separately. To date, after 125 days, the results of this experiment are as follows: After 70 days 7 of the animals in Group I had died, 4 of which were stiff and the survivors were stiff. Two of the latter died 10 days later. The remaining animal was changed to the stock diet and is showing some recovery. In Group II, 6 animals were dead within 70 days, all stiff. The survivors were stiff. One of these died 20 days later. The remaining animals are still alive, 2 of which are showing some recovery as a result of a change to the stock diet. In Group III, 8 animals still survive with no signs of stiffness. Several have more than doubled in weight. The other 2 were killed for autopsy at a time when they were apparently normal. No gross lesions were found. Two of the animals in Group IV died early in the experiment without stiffness. The remainder have continued to grow rapidly and are apparently normal. It is clear that the replacement of the cod liver oil by an AD concentrate has greatly prolonged the survival period on the synthetic diet. In general, it has at least greatly delayed the onset of the stiffness and presumably the onset of the muscle lesions which apparently precede the stiffness, though not preventing the eventual development of the lesions in the few cases (rabbits) examined.

It is tentatively suggested that some factor in the cod liver oil, primarily in the saponifiable fraction, is concerned in the production of the muscle lesions and the other pathological symptoms observed in herbivorous animals fed a synthetic diet. The fact that no deleterious effects have been observed from the same level of cod liver oil when added to a stock diet, may mean that the effects are the combined result of the oil and some other factor peculiar to the synthetic diet, or it may be that a toxic effect of the oil is partially counteracted by some ingredient of the stock diet of natural foods. Our earlier results showed that rabbits which had become stiff frequently recovered when changed to a diet of alfalfa and that the inclusion of alfalfa in the synthetic diet had some protective influence.[4]

The literature contains several reports of deleterious effects of cod liver oil on herbivora. Slagsvold[5] reports a poisoning of cattle by cod liver oil, with stiffness and muscle lesions as a characteristic symptom. Wahlin[6] found degenerative changes in the cardiac and skeletal muscles of guinea pigs which he attributed to cod liver oil. Golding[7] showed that cod liver oil has a specific effect not shown by other oils in lowering the percentage of fat in cow's milk. This finding has been confirmed by several others. Of special interest in connection with our work, is Golding's findings that the unsaponifiable fraction of cod liver oil was without influence.

The significance of our results will become much clearer when the experiments now in progress are finished and when the histological examination of the some 90 animals which we have had on these various experiments are completed. We are aware that muscle lesions have been reported as an accompaniment of various diseases and dietary deficiencies, and that much further study is needed to work out the specific relationships involved in our findings.

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[4] McCay, C. M., Madsen, L. L., and Maynard, L. A., *J. Biol. Chem., Proc.*, 1933, **100**, lxviii.

[5] Slagsvold, L., *Norsk. Vet. Tids.*, 1925, **6**, 161. *Abs. J. A. V. M. A.*, 1925, **68**, 236.

[6] Wahlin, B., *Acta. Med. Scand.*, 1931, **74**, 430.

[7] Golding, J., *Proc. World's Dairy Congress*, 1928.

SECRETARY'S ANNUAL REPORT

April 1, 1932 to April 1, 1933

Standards of Publication Changed. The Council deemed it advisable to increase standards of acceptance of manuscripts, by the adoption of the following policy:

> *"The purpose of the* PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE *is primarily the prompt publication of information that will be of value to scientists. The establishment of claims for priority through publication in the* PROCEEDINGS *is of secondary importance. Authors should, therefore, present their results in such a way that they may be intelligently criticised by the readers of the* PROCEEDINGS. *The experiments on which conclusions are based should be described in sufficient detail to present a clear indication of methods, procedures, apparatus and materials used, and, whenever possible, an illustrative experiment should be described in detail."*

Cost of Publication. The Council, anxious to reduce costs to authors, authorized the use of interest of the Endowment and Surplus Funds. This permits a reduction of forty per cent below cost on text and tables, and twenty-five per cent reduction on plates. *No free reprints,* however, to be given to authors.

There were 613 manuscripts published during the fiscal year, of which 54% were preliminary, 46% complete.

The Council elected Dr. E. B. Wilson to honorary membership.

MEMBERSHIP

Members, March 31, 1932	1128	
Elected during year	25	
Honorary Members	11	
Total		1164
Resignations, year ending March 31, 1933	13	
Deaths, year ending March 31, 1933	4	
Arrears	1	18
Total Membership, March 31, 1933		1146

	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933
Membership	627	692	779	851	936	986	1026	1062	1138	1146

SUBSCRIPTIONS

Subscriptions, March 31, 1932	408	
Subscriptions, March 31, 1933		417

Total No. of PROCEEDINGS distributed 3/31/32.. 1546
Total No. of PROCEEDINGS distributed 3/31/33.. 1563

OFFICERS elected April 19, 1933

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Vice-President: E. L. Opie.
Secretary-Treasurer: A. J. Goldforb.
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Past Presidents (By constitution): E. B. Wilson, Simon Flexner, F. S. Lee, T. H. Morgan, James Ewing, W. J. Gies, Gary Calkins, G. B. Wallace, J. W. Jobling, S. R. Benedict, Peyton Rous, D. J. Edwards.
Chairmen of Sections (See Sections).

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From Other Sections: T. Addis, W. R. Bloor, W. T. Longcope, A. N. Richards, C. J. Wiggers.

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Financial Committee: W. J. Gies, W. H. Park, A. J. Goldforb.

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1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
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1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin

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1926-27	J. W. Jobling	S. R. Benedict	A. J. Goldforb
1927-28	S. R. Benedict	P. Rous	A. J. Goldforb
1928-29	S. R. Benedict	P. Rous	A. J. Goldforb
1929-30	P. Rous	D. Marine	A. J. Goldforb
1930-31	P. Rous	D. J. Edwards	A. J. Goldforb
1931-32	D. J. Edwards	A. R. Dochez	A. J. Goldforb
1932-33	A. R. Dochez	E. L. Opie	A. J. Goldforb

SECTIONS

Cleveland, Ohio

Chairman: N. C. Wetzel. Secretary: A. R. Moritz. Members: 35.

Meetings: Western Reserve University, December 9, 1932.
Western Reserve University, January 13, 1933.
Western Reserve University, February 10, 1933.
Western Reserve University, March 10, 1933.
Western Reserve University, April 14, 1933.
Western Reserve University, May 12, 1933.

Illinois

Chairman: Lloyd Arnold. Secretary: W. L. Palmer. Members: 108.

Meetings: Northwestern University Medical School, October 18, 1932.
University of Chicago, December 16, 1932.
University of Illinois Medical School, February 14, 1933.
Northwestern University Medical School, March 14, 1933.
University of Chicago, May 3, 1933.

Iowa

Chairman: P. C. Jeans. Secretary: W. F. Loehwing. Members: 33.

Meetings: State University of Iowa, October 20, 1932.
State University of Iowa, December 8, 1932.
State University of Iowa, February 23, 1933.
State University of Iowa, May 12, 1933.

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 46.

Meetings: University of Minnesota, November 16, 1932.
University of Minnesota, February 15, 1933.
University of Minnesota, March 22, 1933.
University of Minnesota, May 24, 1933.

Missouri

Chairman: D. P. Barr. Secretary: E. A. Doisy. Members: 44.
Meetings: Washington University Medical School, November 9, 1932.
St. Louis University Medical School, December 14, 1932.
Washington University Medical School, February 8, 1933.
St. Louis University Medical School, May 10, 1933.

New York

Chairman: A. R. Dochez. Secretary: A. J. Goldforb. Members: 632.
Meetings: New York Academy of Medicine, October 19, 1932.
New York Academy of Medicine, November 16, 1932.
New York Academy of Medicine, December 21, 1932.
New York Academy of Medicine, January 18, 1933.
New York Academy of Medicine, February 15, 1933.
New York Academy of Medicine, March 15, 1933.
New York Academy of Medicine, April 19, 1933.
New York Academy of Medicine, May 17, 1933.

Pacific Coast

Chairman: C. L. A. Schmidt. Secretary: M. L. Tainter. Members: 97.
Meetings: Stanford University, October 15, 1932.
University of California Medical School, December 14, 1932.
Stanford University Medical School, February 8, 1933.
Mount Zion Hospital, March 15, 1933.
University of California, April 22, 1933.

Peiping (China)

Chairman: C. M. Van Allen. Secretary: S. H. Liu. Members: 28.
Meetings: Peiping Union Medical College, November 10, 1932.
Peiping Union Medical College, February 22, 1933.

Southern

Chairman: E. C. Faust. Secretary: C. F. Craig. Members: 35.
Meetings: Tulane University Medical School, November 16, 1932.
Tulane University Medical School, January 20, 1933.
Tulane University Medical School, May 19, 1933.

Western New York

Chairman: F. P. Knowlton. Secretary: W. S. Root. Members: 49.
Meetings: Clifton Springs Sanitarium, October 22, 1932.
University of Buffalo, December 17, 1932.
University of Rochester, February 18, 1933.
Cornell University, May 20, 1933.

Wisconsin

Chairman: B. M. Duggar. Secretary: E. L. Sevringhaus. Members: 28.
Meetings: University of Wisconsin, May 8, 1933.

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: C. G. Bull, G. Lusk, H. G. Mehrtens, W. Ophüls, F. P. Underhill, and C. S. Williamson.

GIFTS

The Secretary wishes to acknowledge, with the thanks of the Council, the gift of back numbers from Doctors A. F. Hess, J. G. Hopkins, and G. G. Scott.

A. J. GOLDFORB,
Secretary.

TREASURER'S REPORT

April 1, 1932 to April 1, 1933

RECEIPTS

Balance on hand, April 1, 1932		$ 2,993.00
Income, 1932-33:		
Dues	$ 4,530.05	
Reprints	2,646.56	
Excess space	1,879.16	
Cuts	468.94	
Subscriptions	2,492.63	
Back Numbers Sold	299.42	
Bank Interest and Miscellaneous	47.36	
Total Annual Income		$12,364.12
Total Cash Available April 1, 1932-April 1, 1933		$15,357.12

DISBURSEMENTS

Publication Cost of PROCEEDINGS:		
Printing	$ 6,337.80	
Reprints	2,748.01	
Cuts	430.09	
		$ 9,515.90
Administrative expenses:		
Office Supplies, Postage and Telegrams	$ 351.92	
Salaries	2,080.00	
Storage and Insurance	50.35	
Miscellaneous	216.25	
Refunds	10.95	
		$ 2,709.47
Total Annual Disbursements		$12,225.37
Cash Balance, April 1, 1933		3,131.75
Total		$15,357.12

SUMMARY

Income (net)	$12,364.12
Disbursements (net)	12,225.37
Surplus for year	$ 138.75

FUNDS

Endowment Fund

April 1, 1932	$13,339.71	
Contributions to April 1, 1933	5.00	
Interest to April 1, 1933	671.31	
Total		$14,016.02

Invested in N. Y. Title and Mortgage Co.	$ 6,000.00	
Invested in Title Guar. and Trust Co.	2,500.00	
Invested in Lawyers Mortgage Co.	1,500.00	
Invested in Industrial Bonds	3,055.58	
Invested in R. R. Coop. Bldg. and Loan	960.44	
		$14,016.02

Special Surplus Fund

April 1, 1932	$ 7,237.94	
Interest to April 1, 1933	340.14	
		$ 7,578.08

Invested in Title Guarantee and Trust	$ 2,900.00	
Invested in R. R. Coop. Bldg. and Loan	2,545.11	
Invested in Industrial Bonds	2,132.97	
		$ 7,578.08

Life Membership Fund

Invested in R. R. Coop. Bldg. and Loan	$ 75.00

MEMO

Bills payable	None
Bills receivable	$ 1,472.19

Auditors' Report

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We are convinced that the records of financial transactions are in good order and accurately kept.

A. M. PAPPENHEIMER
W. J. MACNEAL.

April 13, 1933.

MEMBERS' LIST (Alphabetical)

HONORARY MEMBERS

Councilman, William T.	Harvard University
Halliburton, W. D.	London, England
Howell, William H.	Johns Hopkins University
Lombard, Warren P.	University of Michigan
Porter, William	Harvard University
Reichert, E. T.	University of Pennsylvania
Richet, Charles	Paris, France
Schaffer, E. S.	Edinburgh, Scotland
Von Muller, Friedrich	Munich, Germany
Welch, W. H.	Johns Hopkins University
Wilson, Edmund B.	Columbia University

MEMBERS

Abbott, Alexander C.	University of Pennsylvania
Abel, John J.	Johns Hopkins University
Adams, A. Elizabeth	Mount Holyoke College
Adams, William E.	University of Chicago
Addis, Thomas	Medical School, Stanford University
Adler, Herman M.	Berkeley, California
Adolph, E. F.	University of Rochester Medical School
Adolph, W. H.	Peiping, China
Albus, William R.	American Type Culture Collection, Chicago
Alexander, Harry L.	Washington University
Allen, Bennet M.	University of California
Allen, Edgar	University of Missouri
Allen, Ezra	N. Y. Homeopathic Medical College
Allen, William F.	University of Oregon
Alles, G. A.	Los Angeles, California
Alsberg, Carl L.	Stanford University
Alt, Howard L.	Northwestern University Medical School
Alvarez, Walter C.	Mayo Clinic
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	University of Tennessee Medical School
Amoss, Harold L.	Duke University Hospital
Andersen, Dorothy H.	College of Physicians and Surgeons
Anderson, H. H.	University of California Medical School
Anderson, John E.	University of Minnesota
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale University
Anderson, William E.	Yale University
Andrews, Edmund	University of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd	University of Illinois

Aronson, J. D.	Henry Phipps Institute
Asdell, S. A.	Cornell University
Asher, Leon	Berne, Switzerland
Ashman, Richard	Louisiana State Univ. Medical Center
Atchley, D. W.	Presbyterian Hospital, New York City
Atwell, Wayne J.	University of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis University
Austin, J. Harold	University of Pennsylvania
Austin, W. C.	Loyola University
Avery, O. T.	Rockefeller Institute, N. Y. City
Babkin, Boris P.	McGill University
Bachem, Albert	University of Illinois Medical College
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Medical School
Baitsell, George A.	Yale University
Bakwin, Harry	N. Y. University and Bellevue Medical College
Baldridge, Clarence W.	State University of Iowa
Baldwin, W. Manning	Albany Medical College
Ball, G. H.	University of California, Los Angeles
Banta, A. M.	Brown University
Banting, Frederick G.	University of Toronto
Barach, Alvin L.	College of Physicians and Surgeons
Barber, W. Howard	New York University Med. School
Barbour, Henry G.	Yale University
Bardeen, Charles R.	University of Wisconsin
Barlow, Orphus W.	Western Reserve University
Barnett, George D.	Stanford University
Barr, David P.	Washington University
Barron, E. S. G.	University of Chicago
Bass, Charles	Tulane University
Bast, T. H.	University of Wisconsin
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford University
Bayne-Jones, S.	National Research Council, Washington, D. C.
Bazett, H. C.	University of Pennsylvania
Beard, H. H.	Louisiana State Univ. Medical Center
Beck, Claude S.	Western Reserve University
Becker, E. R.	Iowa State College
Beckman, Harry	Marquette University Medical School
Beckwith, T. D.	University of California, Los Angeles
Behre, Jeannette A.	Cornell University Medical College
Belding, David L.	Boston University
Bellamy, Arthur W.	University of California, Los Angeles
Benedict, S. R.	Cornell University Medical College, N. Y. City
Benton, Anne G.	Vassar College

Berg, B. N.	Columbia University
Berg, William N.	Berg Biological Laboratory, N. Y. City
Bergeim, Olaf	University of Illinois
Bergey, David H.	National Drug Co., Philadelphia
Berglund, Hilding	Stockholm, Sweden
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Berry, George P.	University of Rochester Medical School
Beutner, R.	University of Louisville
Bieter, Raymond N.	University of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C.	Western Reserve University
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	Paris, France
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	University of California
Blackfan, K. D.	Harvard Medical School
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale University
Blakeslee, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt University Medical School
Blatherwick, Norman R.	Metropolitan Life Insurance Co., New York City
Blau, Nathan F.	Cornell University Medical College
Blinks, L. R.	Rockefeller Institute
Bliss, Sidney	Tulane University
Bloom, William	University of Chicago
Bloomfield, A. L.	Stanford University Medical School
Bloor, W. R.	University of Rochester
Blum, Harold F.	University of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bodansky, A.	New York City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodine, J. H.	State University of Iowa
Bodo, Richard	N. Y. University and Bellevue Med. College
Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	University of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Presbyterian Hospital, New York City
Bowen, B. D.	Buffalo General Hospital
Boyd, Julian D.	State University of Iowa
Boyd, Theo. E.	Loyola University
Boyden, E. A.	University of Minnesota Medical School
Bradley, H. C.	University of Wisconsin
Brand, Erwin	New York State Psychiatric Institute
Branham, Sara E.	University of Chicago
Brewer, Robert K.	Syracuse University
Briggs, A. P.	St. Louis University
Brodie, Maurice	McGill University
Bronfenbrenner, J.	Washington University
Bronk, D. W.	University of Pennsylvania
Brooks, Clyde	Louisiana State University Medical Center

Brooks, Harlow	New York University
Brooks, Matilda M.	University of California
Brooks, S. C.	University of California
Broun, G. O.	St. Louis University Medical School
Brown, E. D.	University of Minnesota
Brown, J. Howard	Johns Hopkins University
Brown, John B.	Ohio State University
Brown, L. A.	Transylvania College, Lexington, Ky.
Brown, Wade H.	Rockefeller Institute, N. Y. City
Buchanan, Robert E.	Iowa State College
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Buell, Mary V.	Johns Hopkins University
Bulger, H. A.	Washington University
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	University of Wisconsin
Bunting, R. W.	University of Michigan
Burch, John C.	Vanderbilt University Med. School
Burget, G. E.	University of Oregon Medical School
Burky, Earl L.	Johns Hopkins Hospital
Burnett, Theodore C.	University of California
Burns, Robert K., Jr.	University of Rochester
Burr, Harold S.	Yale University
Burrows, M. T.	Pasadena, Calif.
Byrne, Joseph	Fordham University
Calkins, Gary N.	Columbia University
Cannan, Robert K.	N. Y. University and Bellevue Med. College
Cannon, Paul R.	University of Chicago
Cannon, Walter B.	Harvard Medical School
Carlson, A. J.	University of Chicago
Carmichael, E. B.	University of Alabama School of Medicine
Carruthers, Albert	Peiping Union Medical College
Carter, Edward P.	Johns Hopkins Hospital
Casey, Albert E.	Rockefeller Institute
Cash, James R.	University of Virginia
Cattell, McKeen	Cornell University Medical College
Caulfield, A. H. W.	University of Toronto
Cecil, R. L.	Cornell University Medical College
Chace, Arthur F.	N. Y. Post-Graduate Medical College
Chaikoff, I. L.	University of California
Chambers, Robert	New York University
Chambers, William H.	Cornell University Medical College
Chang, Hsi Chun	Peiping Union Medical College
Chang, Hsiao-Chien	Peiping Union Medical College
Cheer, S. N.	Peiping Union Medical College
Chen, K. K.	Eli Lilly and Co., Indianapolis
Cheney, R. H.	Long Island University
Chidester, F. E.	University of West Virginia
Child, C. M.	University of Chicago
Chittenden, R. H.	Yale University

Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	University of Michigan
Churchman, John W.	Cornell University Medical School
Clark, A. J.	Edinburgh, Scotland
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	University of Wisconsin
Clarke, Hans T.	College of Physicians and Surgeons
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clawson, Benjamin J.	University of Minnesota
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Clifton, Charles E.	Stanford University
Coca, A. F.	Oradell, N. J.
Coghill, G. E.	Wistar Institute
Cohen, Barnett	Johns Hopkins Medical School
Cohen, Martin	N. Y. Post-Graduate Medical School
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, L. J.	University of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers University
Coleman, Warren	New York University Medical College
Collens, William S.	Brooklyn Jewish Hospital
Collett, Mary E.	Western Reserve University
Collier, William D.	St. Louis University
Collins, H. H.	Crafton, Pa.
Collip, J. B.	McGill University
Conklin, E. G.	Princeton University
Connor, Charles L.	University of California Medical School
Cook, Donald H.	School of Tropical Medicine, San Juan, Porto Rico
Cook, S. F.	University of California
Cooke, J. V.	Washington University
Coombs, Helen C.	New York Homeopathic Medical College
Copenhaver, W. M.	Columbia University
Cori, Carl F.	Washington University
Corley, Ralph C.	Purdue University
Corner, George W.	University of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Coulter, Calvin B.	Columbia University
Cowdry, E. V.	Washington University
Cowgill, George R.	Yale University
Cowie, D. M.	University of Michigan
Craig, C. F.	Tulane University
Crampton, C. Ward	N. Y. Post-Graduate Medical School
Crandall, L. A., Jr.	Northwestern University
Crile, George W.	Western Reserve University
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Crozier, W. J.	Harvard University
Cruickshank, E. W. H.	Dalhousie University, Halifax
Csonka, F. A.	U. S. Dept. of Agriculture, Washington, D. C.
Cullen, Glenn E.	Children's Hospital, Cincinnati, O.

Cummins, Harold	Tulane University
Cunningham, R. S.	Vanderbilt University
Curtis, G. M.	Ohio State University
Curtis, Maynie R.	Columbia University
Cushing, Harvey W.	Harvard Medical School
Cutler, Elliott C.	Peter Bent Brigham Hospital, Boston

Dack, Gail M.	University of Chicago
Dakin, H. D.	Ossining, N. Y.
Danforth, Charles H.	Stanford University
Daniel, J. Frank	University of California
Daniels, Amy L.	University of Iowa
Daniels, Farrington	University of Wisconsin
Danzer, Charles S.	N. Y. Homeopathic Medical College
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davenport, H. A.	Northwestern University Medical School
Davies, H. Whitridge	The University, Sydney, Australia
Davis, D. J.	Wilmette, Illinois
Davis, Nelson C.	Rockefeller Foundation
Dawson, James A.	College of the City of New York
Dawson, W. T.	University of Texas, Med. School
Day, A. A.	Northwestern University Medical School
De Eds, Floyd	Stanford University Medical School
DeGraff, A. C.	N. Y. University Medical School
DeRenyi, G. S.	University of Pennsylvania
Derick, C. L.	Peter Bent Brigham Hospital, Boston
Detwiler, S. R.	Columbia University
Deuel, Harry J., Jr.	University of Southern California Medical School
Dickson, E. C.	Stanford University Medical School
Dienes, Louis	Massachusetts General Hospital, Boston
Dieuaide, Francis R.	Peiping Union Medical College, China
Doan, Charles A.	Ohio State University
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford University
Doisy, Edward A.	St. Louis University
Dolley, W. L., Jr.	University of Buffalo
Donaldson, H. H.	Wistar Institute, Philadelphia
Donaldson, J. C.	University of Pittsburgh
Dooley, M. S.	Syracuse University
Downs, W. G., Jr.	Chicago Medical School
Drabkin, D. L.	University of Pennsylvania
Dragstedt, Carl A.	Northwestern University
Dragstedt, Lester R.	University of Chicago
Draper, George W.	Columbia University
Draper, John W.	New York City
Dresbach, M.	Albany Medical College
Drury, D. R.	University of Southern California Medical School
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell University Medical College
Duggar, B. M.	University of Wisconsin

Dunn, L. C.	Columbia University
Dunn, Max	University of California, Los Angeles
Durrant, Edwin P.	Ohio State University
Dusser de Barenne, J. G.	Yale University
Dutcher, R. Adams	Pennsylvania State College
Duval, C. W.	Tulane University
Du Vigneaud, Vincent	University of Illinois
Dye, Joseph A.	Cornell University Medical School
Eberson, Frederick	University of California Medical School
Ecker, E. E.	Western Reserve University
Eckles, C. H.	University of Minnesota
Eckstein, Henry C.	University of Michigan
Eddy, Walter H.	Columbia University
Edmunds, C. W.	University of Michigan
Edwards, D. J.	Cornell University Medical College
Edwards, John G.	University of Buffalo
Eggston, Andrew A.	Manhattan Eye, Ear, Nose and Throat Hospital, N. Y. City
Eisberg, Harry B.	N. Y. University and Bellevue Medical College
Ellis, Max M.	University of Missouri
Ellsworth, R. M.	Johns Hopkins University
Elsberg, Charles A.	Neurological Institute, N. Y. City
Elser, W. J.	Cornell University Medical College
Emery, F. E.	University of Buffalo
Engle, E. T.	Columbia University
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erdmann, Rhoda	University of Berlin, Germany
Erlanger, Joseph	Washington University
Ernstene, Arthur C.	Cleveland Clinic
Essex, Hiram E.	Mayo Clinic
Evans, Herbert M.	Rockefeller Institute
Everett, M. R.	University of Oklahoma Med. School
Ewing, James	Cornell University Medical College
Eyster, J. A. E.	University of Wisconsin
Faber, Harold K.	Stanford University Medical School
Fahr, George	University of Minnesota
Falk, I. S.	Washington, D. C.
Falk, K. George	Harriman Laboratories, N. Y. City
Falls, Frederick H.	University of Illinois
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Farmer, Chester	Northwestern University
Faust, Ernest C.	Tulane University
Feil, Harold	Western Reserve University
Fenn, Wallace O.	University of Rochester Medical School
Ferraro, Armando	N. Y. State Psychiatric Institute
Ferry, R. M.	Harvard University
Field, Cyrus W.	Westfield, New York
Field, John, II	Stanford University

Fine, M. S.	Battle Creek, Mich.
Firor, W. M.	Johns Hopkins University
Fischer, Albert	Kopenhagen, Denmark
Fischer, Martin H.	University of Cincinnati
Fishberg, Ella H.	Beth Israel Hospital, New York City
Fisher, Nelson F.	St. Luke's Hospital, Chicago
Fitch, C. P.	University of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
Fitzgerald, J. G.	University of Toronto
Fleisher, Moyer S.	St. Louis University
Flexner, Simon	Rockefeller Institute, N. Y. City
Flinn, Frederick B.	Columbia University
Florence, Laura	New York Homeopathic Medical School
Fluhmann, Charles F.	Stanford University Medical School
Forbes, Henry	Milton, Mass.
Forkner, Claude E.	Peiping Union Medical College
Fortuyn, A. B. D.	Peiping Union Medical College
Foster, Goodwin L.	Columbia University
Foster, Nellis B.	New York Hospital
Frank, Robert T.	Mt. Sinai Hospital
Frazier, Chester N.	Peiping Union Medical College
Fred, E. B.	University of Wisconsin
Freedlander, S. O.	Western Reserve University
Freedman, Louis	Rensselaer, N. Y.
Freeman, R. G., Jr.	Columbia University
Freund, Jules	Cornell University Medical College
Fridericia, L. S.	University of Copenhagen, Denmark
Friedemann, T. E.	University of Chicago
Frobisher, Martin, Jr.	Baltimore, Md.
Fry, Henry J.	New York University
Fulmer, Ellis I.	Iowa State College
Fulton, John F.	Yale Medical School
Funk, Casimir	Paris, France
Furth, Jacob	Cornell University Medical College

Gaebler, O. H.	Henry Ford Hospital, Detroit
Gage, I. M.	Tulane University
Gager, C. Stuart	Brooklyn Botanic Garden, Brooklyn, N. Y.
Gamble, James L.	Harvard University
Garbat, Abraham L.	Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.	Saranac Lab. for Study of Tuberculosis
Garrey, Walter E.	Vanderbilt University
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	New York City
Gates, Frederick L.	Cambridge, Mass.
Gay, F. P.	Columbia University
Geiling, E. M. K.	Johns Hopkins Medical School
Gelfan, Samuel	University of Alberta, Canada
Gellhorn, Ernst	University of Illinois Medical College
Gerard, R. W.	University of Chicago

Gesell, Robert A.	University of Michigan
Gettler, A. O.	University and Bellevue Medical College
Geyelin, H. R.	Columbia University
Gibson, R. B.	University of Iowa
Gies, William J.	Columbia University
Gilbert, E. M.	University of Wisconsin
Gilchrist, Francis G.	Pomona College
Gilligan, Dorothy R.	Beth Israel Hospital, Boston
Gilson, A. S., Jr.	Washington University Medical School
Githens, T. S.	Mulford Company, Philadelphia, Pa.
Givens, Maurice H.	Northwestern Yeast Co., Chicago
Glaser, Otto	Amherst College
Gold, Harry	Cornell University Medical College
Goldberg, S. A.	Presbyterian Hospital, Newark, N. J.
Goldblatt, Harry	Western Reserve University
Goldbloom, A. Allen	Beth Israel Hospital, N. Y. City
Goldforb, A. J.	College of the City of New York
Goldschmidt, Samuel	University of Pennsylvania
Goodner, Kenneth	Rockefeller Institute
Gortner, R. A.	University of Minnesota
Gottesman, J. Marmorston	Montefiore Hospital, N. Y. City
Gould, Harley N.	Tulane University
Graham, Evarts A.	Washington University
Graham, Helen T.	Washington University
Graves, William W.	St. Louis University
Gray, Samuel H.	Jewish Hospital, St. Louis, Mo.
Green, Robert G.	University of Minnesota
Greenberg, David N.	University of California
Greene, Carl H.	Brooklyn, N. Y.
Greenwald, Isidor	N. Y. University and Bellevue Med. College
Greenwood, Alan	University of Edinburgh
Greer, J. R.	University of Chicago
Gregory, Louise H.	Barnard College, Columbia University
Greisheimer, Esther M.	University of Minnesota
Griffith, Fred R., Jr.	University of Buffalo
Griffith, Wendell H.	St. Louis University
Gross, Erwin G.	State University of Iowa
Gross, Louis	Mt. Sinai Hospital, N. Y. City
Gruber, Charles M.	Jefferson Medical College
Guberlet, J. E.	University of Washington
Guenther, A. E.	University of Nebraska
Gunn, Francis D.	Northwestern University Med. School
Gurchot, Charles	Eldridge, California
Gustavson, R. G.	University of Denver
Guthrie, C. C.	University of Pittsburgh
Guy, Ruth A.	Peiping Union Medical College
Guyer, Michael F.	University of Wisconsin

Hadley, Philip	University of Michigan
Hafkesbring, H. Roberta	Woman's Medical College, Philadelphia

Hagan, William Arthur	Cornell University
Hall, Ernest M.	University of Southern California Med. School
Halsey, John	Tulane University
Halsey, Robert H.	N. Y. Post-Graduate Medical School
Hamburger, W. W.	Rush Medical College
Hamilton, B. K.	Chicago, Illinois
Hammer, B. W.	Iowa State College
Hanger, Franklin M.	Presbyterian Hospital, N. Y. City
Hanke, Martin E.	University of Chicago
Hannon, R. R.	Peiping Union Medical College
Hansmann, G. H.	State University of Iowa
Hanson, Frank B.	Washington University
Hanzlik, P. J.	Stanford University Medical School
Hardesty, Irving	Tulane University
Harris, Isaac F.	Tuckahoe, N. Y.
Harris, Reginald G.	Cold Spring Harbor, N. Y.
Harris, William H.	Tulane University
Harrison, R. G.	Yale University
Harrop, George A., Jr.	Johns Hopkins University
Harrow, Benjamin	College of the City of New York
Hart, Edwin B.	University of Wisconsin
Hart, George H.	University Farm, Davis, California
Hartman, F. A.	University of Buffalo
Hartwell, John A.	Cornell University Medical College
Harvey, E. Newton	Princeton University
Harvey, Samuel C.	Yale University
Hastings, A. Baird	University of Chicago
Hastings, E. G.	University of Wisconsin
Hatcher, R. A.	Cornell University Medical College
Hathaway, Edward S.	Tulane University
Hawk, P. B.	New York City
Hawkins, James A.	U. S. Navy Yards, Washington, D. C.
Hayden, Charles E.	Cornell University
Hayman, J. M., Jr.	Western Reserve University
Haythorn, Samuel R.	Singer Research Lab., Pittsburgh, Pa.
Heft, Hattie L.	Columbia University
Heidelberger, Michael	Presbyterian Hospital, New York City
Heilbrunn, L. V.	University of Pennsylvania
Heinbecker, Peter	Washington University
Helff, O. M.	New York University
Helmholz, Henry R.	Mayo Clinic
Hench, Philip S.	Mayo Clinic
Hendrix, B. M.	University of Texas
Henrici, Arthur T.	University of Minnesota
Henshaw, P. S.	Memorial Hospital, New York City
Herrmann, G. R.	John Sealy Hospital, Galveston, Texas
Herrold, Russell D.	University of Illinois Medical School
Hertzman, A. B.	St. Louis University
Hess, Alfred F.	College of Physicians and Surgeons
Heymans, J. F.	U. de Gand, Belgium

Higgins, George M.	Mayo Clinic
Himwich, H. E.	Yale University
Hines, H. M.	State University of Iowa
Hinman, Frank	University of California Med. School
Hinrichs, Marie Agnes	University of Chicago
Hinsey, J. C.	Stanford University
Hinshaw, H. C.	Upper Darby, Pa.
Hirschfelder, Arthur	University of Minnesota
Hisaw, F. L.	University of Wisconsin
Hitchcock, Fred A.	Ohio State University
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